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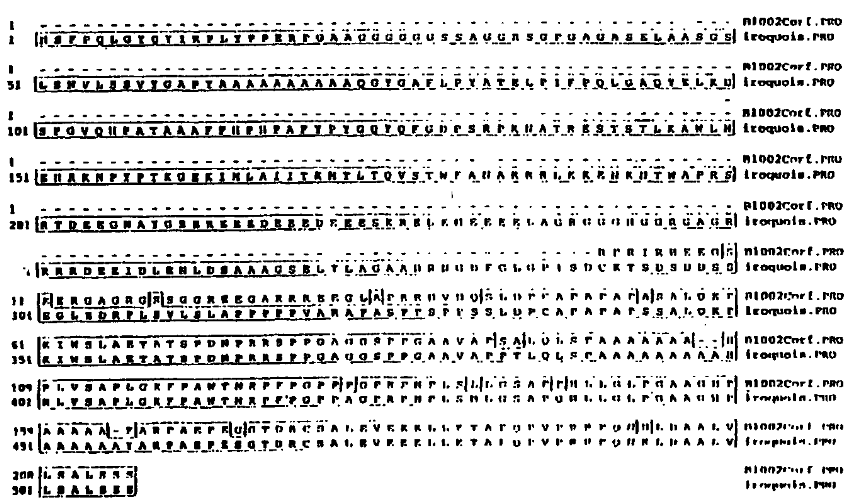
(54) Title: COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER



(57) Abstract: Compositions and methods for the therapy and diagnosis of cancer, such as breast cancer, are disclosed. Compositions may comprise one or more breast tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a breast tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as breast cancer. Diagnostic methods based on detecting a breast tumor protein, or mRNA encoding such a protein, in a sample are also provided.

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COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to therapy and diagnosis of cancer, such as breast cancer. The invention is more specifically related to polypeptides comprising at least a portion of a breast tumor protein, and to polynucleotides encoding such polypeptides. Such polypeptides and polynucleotides may be used in vaccines and pharmaceutical compositions for prevention and treatment of breast cancer, and for the diagnosis and monitoring of such cancers.

BACKGROUND OF THE INVENTION

Breast cancer is a significant health problem for women in the United States and throughout the world. Although advances have been made in detection and treatment of the disease, breast cancer remains the second leading cause of cancer-related deaths in women, affecting more than 180,000 women in the United States each year. For women in North America, the life-time odds of getting breast cancer are now one in eight.

No vaccine or other universally successful method for the prevention or treatment of breast cancer is currently available. Management of the disease currently relies on a combination of early diagnosis (through routine breast screening procedures) and aggressive treatment, which may include one or more of a variety of treatments such as surgery, radiotherapy, chemotherapy and hormone therapy. The course of treatment for a particular breast cancer is often selected based on a variety of prognostic parameters, including an analysis of specific tumor markers. *See, e.g.,* Porter-Jordan and Lippman, *Breast Cancer 8*:73-100, 1994. However, the use of established markers often leads to a result that is difficult to interpret, and the high mortality observed in breast cancer patients indicates that improvements are needed in the treatment, diagnosis and prevention of the disease.

Immunotherapies have the potential to substantially improve breast cancer treatment and survival. Such therapies may involve the generation or

enhancement of an immune response to a breast tumor antigen. However, to date, relatively few breast tumor antigens are known and the generation of an immune response against such antigens has not been shown to be therapeutically beneficial.

Accordingly, there is a need in the art for improved methods for identifying breast tumor antigens and for using such antigens in the diagnosis and therapy of breast cancer. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for the diagnosis and therapy of cancer, such as breast cancer. In one aspect, the present invention provides polypeptides comprising at least a portion of a breast tumor protein, or a variant thereof. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments, the polypeptide comprises a sequence that is encoded by a polynucleotide sequence selected from the group consisting of sequences recited in SEQ ID NOs:1-125, variants of such sequences and complements of such sequences. One such polypeptide comprises a sequence recited in SEQ ID NO:126, or a variant thereof that is at least 90% identical to SEQ ID NO:126.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 9, preferably at least 15, amino acid residues of a breast tumor protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

Within other aspects, the present invention provides pharmaceutical compositions comprising a polypeptide or polynucleotide as described above and a physiologically acceptable carrier.

Within a related aspect of the present invention, vaccines are provided. Such vaccines comprise a polypeptide or polynucleotide as described above and a non-specific immune response enhancer.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to a breast tumor protein; and (b) a physiologically acceptable carrier.

Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient. Antigen presenting cells include dendritic cells, macrophages and B cells.

Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a non-specific immune response enhancer.

The present invention further provides, in other aspects, fusion proteins that comprise at least one polypeptide as described above, as well as polynucleotides encoding such fusion proteins.

Within related aspects, pharmaceutical compositions comprising a fusion protein, or a polynucleotide encoding a fusion protein, in combination with a physiologically acceptable carrier are provided.

Vaccines are further provided, within other aspects, that comprise a fusion protein or a polynucleotide encoding a fusion protein in combination with a non-specific immune response enhancer.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as recited above.

The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a breast tumor protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated as described above.

Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for a breast tumor protein, comprising contacting T cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that expresses such a polypeptide; under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population as described above.

The present invention further provides methods for inhibiting the development of a cancer in a patient, comprising the steps of: (a) incubating $CD4^+$ and/or $CD8^+$ T cells isolated from a patient with one or more of: (i) a polypeptide comprising at least an immunogenic portion of a breast tumor protein; (ii) a polynucleotide encoding such a polypeptide; and (iii) an antigen-presenting cell that expresses such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

Within further aspects, the present invention provides methods for determining the presence or absence of a cancer in a patient, comprising (a) contacting a biological sample obtained from a patient with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within preferred embodiments, the binding agent is an antibody, more preferably a monoclonal antibody. The cancer may be breast cancer.

The present invention also provides, within other aspects, methods for monitoring the progression of a cancer in a patient. Such methods comprise the steps of: (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a polypeptide as recited above; (b) detecting in the

sample an amount of polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polypeptide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

The present invention further provides, within other aspects, methods for determining the presence or absence of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within certain embodiments, the amount of mRNA is detected via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a complement of such a polynucleotide.

In related aspects, methods are provided for monitoring the progression of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein; (b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polynucleotide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

Within further aspects, the present invention provides antibodies, such as monoclonal antibodies, that bind to a polypeptide as described above, as well as

diagnostic kits comprising such antibodies. Diagnostic kits comprising one or more oligonucleotide probes or primers as described above are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 represents a sequence alignment between the representative breast tumor polypeptide B1002C (SEQ ID NO:126) and the mouse iroquois homeobox protein 3.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for using the compositions, for example in the therapy and diagnosis of cancer, such as breast cancer. Certain illustrative compositions described herein include breast tumor polypeptides, polynucleotides encoding such polypeptides, binding agents such as antibodies, antigen presenting cells (APCs) and/or immune system cells (*e.g.*, T cells). A "breast tumor protein," as the term is used herein, refers generally to a protein that is expressed in breast tumor cells at a level that is at least two fold, and preferably at least five fold, greater than the level of expression in a normal tissue, as determined using a representative assay provided herein. Certain breast tumor proteins are tumor proteins that react detectably (within an immunoassay, such as an ELISA or Western blot) with antisera of a patient afflicted with breast cancer.

Therefore, in accordance with the above, and as described further below, the present invention provides illustrative polynucleotide compositions having sequences set forth in SEQ ID NO:1-125, illustrative polypeptide compositions having amino acid sequences set forth in SEQ ID NO:126, antibody compositions capable of binding such polypeptides, and numerous additional embodiments employing such compositions, for example in the detection, diagnosis and/or therapy of human breast cancer.

POLYNUCLEOTIDE COMPOSITIONS

As used herein, the terms "DNA segment" and "polynucleotide" refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a polypeptide refers to a DNA segment that contains one or more coding sequences yet is substantially isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained. Included within the terms "DNA segment" and "polynucleotide" are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

As will be understood by those skilled in the art, the DNA segments of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

"Isolated," as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the DNA segment does not contain large portions of unrelated coding DNA, such as large chromosomal fragments or other functional genes or polypeptide coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

As will be recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a breast tumor protein or a portion thereof) or may comprise a variant, or a biological or antigenic functional equivalent of such a sequence.

Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native tumor protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. The term "variants" also encompasses homologous genes of xenogenic origin.

When comparing polynucleotide or polypeptide sequences, two sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS 5*:151-153; Myers, E.W. and Muller W. (1988) *CABIOS 4*:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol. 4*:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA 80*:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL.*

Math 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) *Nucl. Acids Res.* 25:3389-3402 and Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference

sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Therefore, the present invention encompasses polynucleotide and polypeptide sequences having substantial identity to the sequences disclosed herein, for example those comprising at least 50% sequence identity, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide or polypeptide sequence of this invention using the methods described herein, (*e.g.*, BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

In additional embodiments, the present invention provides isolated polynucleotides and polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their

overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

In other embodiments, the present invention is directed to polynucleotides that are capable of hybridizing under moderately stringent conditions to a polynucleotide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS.

Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

PROBES AND PRIMERS

In other embodiments of the present invention, the polynucleotide sequences provided herein can be advantageously used as probes or primers for nucleic acid hybridization. As such, it is contemplated that nucleic acid segments that comprise a sequence region of at least about 15 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 15 nucleotide long contiguous sequence disclosed herein will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to a sequence of interest will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are also envisioned, such as the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Polynucleotide molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so (including intermediate lengths as well), identical or complementary to a polynucleotide sequence disclosed herein, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow a gene product, or fragment thereof, to be analyzed, both in diverse cell types and also in various bacterial cells. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 15 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences one wishes to detect.

The use of a hybridization probe of about 15-25 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 15 bases in

length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 25 contiguous nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the sequences disclosed herein. All that is required is to review the sequence set forth in SEQ ID NO:1-125, or to any continuous portion of the sequence, from about 15-25 nucleotides in length up to and including the full length sequence, that one wishes to utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors. For example, one may wish to employ primers from towards the termini of the total sequence.

Small polynucleotide segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U. S. Patent 4,683,202 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

The nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of the entire gene or gene fragments of interest. Depending on the application envisioned, one will typically desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating related sequences.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template, less stringent (reduced stringency) hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ salt conditions such as those of from about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

POLYNUCLEOTIDE IDENTIFICATION AND CHARACTERIZATION

Polynucleotides may be identified, prepared and/or manipulated using any of a variety of well established techniques. For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (*i.e.*, expression that is at least two fold greater in a tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed, for example, using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena *et al.*, *Proc. Natl. Acad. Sci. USA 93*:10614-10619, 1996 and Heller *et al.*, *Proc. Natl. Acad. Sci. USA 94*:2150-2155, 1997). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as breast tumor cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (*e.g.*, a breast tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for

amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia *et al.*, *Nucl. Acids Res. 16*:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known

region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom *et al.*, *PCR Methods Applic. 1*:111-19, 1991) and walking PCR (Parker *et al.*, *Nucl. Acids. Res. 19*:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

POLYNUCLEOTIDE EXPRESSION IN HOST CELLS

In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular

prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 215-223, Horn, T. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. *et al.* (1995) *Science 269*:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, N.Y.) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. *et al.* (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. *et al.* (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out

transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the PBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or PSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of .beta.-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) *J. Biol. Chem. 264*:5503-5509); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel *et al.* (supra) and Grant *et al.* (1987) *Methods Enzymol.* 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J. 6*:307-311. Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. *et al.* (1984) *EMBO J. 3*:1671-1680; Broglie, R. *et al.* (1984) *Science 224*:838-843; and Winter, J. *et al.* (1991) *Results Probl. Cell Differ. 17*:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which the polypeptide of interest may be expressed (Engelhard, E. K. *et al.* (1994) *Proc. Natl. Acad. Sci. 91* :3224-3227).

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) *Proc. Natl. Acad. Sci. 81*:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. *et al.* (1994) *Results Probl. Cell Differ. 20*:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation. glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which

successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. *et al.* (1977) *Cell 11*:223-32) and adenine phosphoribosyltransferase (Lowy, I. *et al.* (1990) *Cell 22*:817-23) genes which can be employed in tk.sup.- or aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. *et al.* (1980) *Proc. Natl. Acad. Sci. 77*:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. et al (1981) *J. Mol. Biol. 150*:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci. 85*:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. *et al.* (1995) *Methods Mol. Biol. 55*:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-

RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. *et al.* (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul. Minn.) and Maddox, D. E. *et al.* (1983; *J. Exp. Med. 158*:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the

encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen. San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. *et al.* (1992, *Prot. Exp. Purif.* *3*:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. *et al.* (1993; *DNA Cell Biol.* *12*:441-453).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) *J. Am. Chem. Soc.* *85*:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

SITE-SPECIFIC MUTAGENESIS

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent polypeptides, through specific mutagenesis of the underlying polynucleotides that encode them. The technique,

well-known to those of skill in the art, further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the antigenicity of a polypeptide vaccine. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25 nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated

sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

POLYNUCLEOTIDE AMPLIFICATION TECHNIQUES

A number of template dependent processes are available to amplify the target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, each of which is incorporated herein by reference in its entirety. Briefly, in PCR™, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates is added to a reaction mixture along with a DNA polymerase (*e.g.*, *Taq* polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, excess primers will bind to the target and to the reaction product and the process is repeated. Preferably reverse transcription and PCR™ amplification procedure may be performed in order to quantify the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Another method for amplification is the ligase chain reaction (referred to as LCR), disclosed in Eur. Pat. Appl. Publ. No. 320,308 (specifically incorporated herein by reference in its entirety). In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCR™, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Patent No. 4,883,750, incorporated herein by reference in its entirety, describes an alternative method of amplification similar to LCR for binding probe pairs to a target sequence.

Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880, incorporated herein by reference in its entirety, may also be used as still another amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a

sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence that can then be detected.

An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[α-thio]triphosphates in one strand of a restriction site (Walker *et al.*, 1992, incorporated herein by reference in its entirety), may also be useful in the amplification of nucleic acids in the present invention.

Strand Displacement Amplification (SDA) is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, *i.e.* nick translation. A similar method, called Repair Chain Reaction (RCR) is another method of amplification which may be useful in the present invention and is involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. A similar approach is used in SDA.

Sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having a 3' and 5' sequences of non-target DNA and an internal or "middle" sequence of the target protein specific RNA is hybridized to DNA which is present in a sample. Upon hybridization, the reaction is treated with RNaseH, and the products of the probe are identified as distinctive products by generating a signal that is released after digestion. The original template is annealed to another cycling probe and the reaction is repeated. Thus, CPR involves amplifying a signal generated by hybridization of a probe to a target gene specific expressed nucleic acid.

Still other amplification methods described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety, may be used in accordance with the present invention. In the former application, "modified" primers are used in a PCR-like, template and enzyme dependent synthesis. The primers may be modified by labeling with a capture moiety (*e.g.*, biotin) and/or a detector moiety (*e.g.*, enzyme). In the latter application, an excess of labeled probes is added to a sample. In the presence of the target sequence, the probe binds and is cleaved catalytically. After cleavage, the

target sequence is released intact to be bound by excess probe. Cleavage of the labeled probe signals the presence of the target sequence.

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh *et al.,* 1989; PCT Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer that has sequences specific to the target sequence. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat-denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into DNA, and transcribed once again with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target-specific sequences.

Eur. Pat. Appl. Publ. No. 329,822, incorporated herein by reference in its entirety, disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention. The ssRNA is a first template for a first primer oligonucleotide, which is elongated by reverse transcriptase (RNA-dependent DNA polymerase). The RNA is then removed from resulting DNA:RNA duplex by the action of ribonuclease H (RNase H, an RNase specific for RNA in a duplex with either DNA or RNA). The resultant ssDNA is a second template for a second primer, which also includes the sequences of an RNA polymerase promoter (exemplified by T7 RNA polymerase) 5' to its homology to its template. This primer is then extended by DNA polymerase (exemplified by the large "Klenow" fragment of *E. coli* DNA polymerase I), resulting as a double-stranded DNA ("dsDNA") molecule, having a sequence identical to that of the original RNA between

the primers and having additionally, at one end, a promoter sequence. This promoter sequence can be used by the appropriate RNA polymerase to make many RNA copies of the DNA. These copies can then re-enter the cycle leading to very swift amplification. With proper choice of enzymes, this amplification can be done isothermally without addition of enzymes at each cycle. Because of the cyclical nature of this process, the starting sequence can be chosen to be in the form of either DNA or RNA.

PCT Intl. Pat. Appl. Publ. No. WO 89/06700, incorporated herein by reference in its entirety, disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic; *i.e.* new templates are not produced from the resultant RNA transcripts. Other amplification methods include "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) which are well-known to those of skill in the art.

Methods based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide (Wu and Dean, 1996, incorporated herein by reference in its entirety), may also be used in the amplification of DNA sequences of the present invention.

BIOLOGICAL FUNCTIONAL EQUIVALENTS

Modification and changes may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a polypeptide with desirable characteristics. As mentioned above, it is often desirable to introduce one or more mutations into a specific polynucleotide sequence. In certain circumstances, the resulting encoded polypeptide sequence is altered by this mutation, or in other cases, the sequence of the polypeptide is unchanged by one or more mutations in the encoding polynucleotide.

When it is desirable to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, second-generation molecule, the amino acid

changes may be achieved by changing one or more of the codons of the encoding DNA sequence, according to Table 1.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

TABLE 1

Amino Acids			Codons					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are:

isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (–0.4); threonine (–0.7); serine (–0.8); tryptophan (–0.9); tyrosine (–1.3); proline (–1.6); histidine (–3.2); glutamate (–3.5); glutamine (–3.5); aspartate (–3.5); asparagine (–3.5); lysine (–3.9); and arginine (–4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.* still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (–0.4); proline (–0.5 ± 1); alanine (–0.5); histidine (–0.5); cysteine (–1.0); methionine (–1.3); valine (–1.5); leucine (–1.8); isoleucine (–1.8); tyrosine (–2.3); phenylalanine (–2.5); tryptophan (–3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those

of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition, any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

IN VIVO POLYNUCLEOTIDE DELIVERY TECHNIQUES

In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells *in vivo*. This may be achieved using any of a variety or well known approaches, several of which are outlined below for the purpose of illustration.

1. ADENOVIRUS

One of the preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a polynucleotide that has been cloned therein in a sense or antisense orientation. Of course, in the context of an antisense construct, expression does not require that the gene product be synthesized.

The expression vector comprises a genetically engineered form of an adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kb, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus and Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement

has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range and high infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are *cis* elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome contain different transcription units that are divided by the onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off (Renan, 1990). The products of the late genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP, (located at 16.8 m.u.) is particularly efficient during the late phase of infection, and all the mRNA's issued from this promoter possess a 5'-tripartite leader (TPL) sequence which makes them preferred mRNA's for translation.

In a current system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible recombination between two proviral vectors, wild-type adenovirus may be generated from this process. Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.*, 1977). Since the E3 region is dispensable from the adenovirus genome (Jones and Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham and Prevec, 1991). In nature, adenovirus can package

approximately 105% of the wild-type genome (Ghosh-Choudhury *et al.*, 1987), providing capacity for about 2 extra kB of DNA. Combined with the approximately 5.5 kB of DNA that is replaceable in the E1 and E3 regions, the maximum capacity of the current adenovirus vector is under 7.5 kB, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-borne cytotoxicity. Also, the replication deficiency of the E1-deleted virus is incomplete. For example, leakage of viral gene expression has been observed with the currently available vectors at high multiplicities of infection (MOI) (Mulligan, 1993).

Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, *e.g.*, Vero cells or other monkey embryonic mesenchymal or epithelial cells. As stated above, the currently preferred helper cell line is 293.

Recently, Racher *et al.* (1995) disclosed improved methods for culturing 293 cells and propagating adenovirus. In one format, natural cell aggregates are grown by inoculating individual cells into 1 liter siliconized spinner flasks (Techne, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and adenovirus added at an MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may

be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain a conditional replication-defective adenovirus vector for use in the present invention, since Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson *et al.* (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, *e.g.*, 10^9-10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch *et al.*, 1963; Top *et al.*, 1971), demonstrating their safety and therapeutic potential as *in vivo* gene transfer vectors.

Adenovirus vectors have been used in eukaryotic gene expression (Levrero *et al.*, 1991; Gomez-Foix *et al.*, 1992) and vaccine development (Grunhaus and Horwitz, 1992; Graham and Prevec, 1992). Recently, animal studies suggested that recombinant adenovirus could be used for gene therapy (Stratford-Perricaudet and Perricaudet, 1991; Stratford-Perricaudet *et al.*, 1990; Rich *et al.*, 1993). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld *et al.*, 1991; Rosenfeld *et al.*, 1992), muscle injection (Ragot *et al.*, 1993),

peripheral intravenous injections (Herz and Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle *et al.*, 1993).

2. RETROVIRUSES

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5′ and 3′ ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

In order to construct a retroviral vector, a nucleic acid encoding one or more oligonucleotide or polynucleotide sequences of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind *et al.*, 1975).

A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes *via* sialoglycoprotein receptors.

A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

3. ADENO-ASSOCIATED VIRUSES

AAV (Ridgeway, 1988; Hermonat and Muzycska, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies are present in 85% of the US human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replications is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of which AAV-2 is the best characterized. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of 20 to 24 nm in diameter (Muzyczka and McLaughlin, 1988).

The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs (FIG. 2). There are two major genes in the AAV genome: *rep* and *cap*. The *rep* gene codes for proteins responsible for viral replications, whereas *cap* codes for capsid protein VP1-3. Each ITR forms a T-shaped hairpin structure. These terminal repeats are the only essential *cis* components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding sequences removed and replaced by the cassette of genes for delivery. Three viral promoters have been identified and named p5, p19, and p40, according to their map position. Transcription from p5 and p19 results in production of rep proteins,

and transcription from p40 produces the capsid proteins (Hermonat and Muzyczka, 1984).

There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector. One is that the requirements for delivering a gene to integrate into the host chromosome are surprisingly few. It is necessary to have the 145-bp ITRs, which are only 6% of the AAV genome. This leaves room in the vector to assemble a 4.5-kb DNA insertion. While this carrying capacity may prevent the AAV from delivering large genes, it is amply suited for delivering the antisense constructs of the present invention.

AAV is also a good choice of delivery vehicles due to its safety. There is a relatively complicated rescue mechanism: not only wild type adenovirus but also AAV genes are required to mobilize rAAV. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimizes immune reactions to viral gene expression, and therefore, rAAV does not evoke an inflammatory response.

4. OTHER VIRAL VECTORS AS EXPRESSION CONSTRUCTS

Other viral vectors may be employed as expression constructs in the present invention for the delivery of oligonucleotide or polynucleotide sequences to a host cell. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Coupar *et al.*, 1988), lentiviruses, polio viruses and herpes viruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar *et al.*, 1988; Horwich *et al.*, 1990).

With the recent recognition of defective hepatitis B viruses, new insight was gained into the structure-function relationship of different viral sequences. *In vitro* studies showed that the virus could retain the ability for helper-dependent packaging and reverse transcription despite the deletion of up to 80% of its genome (Horwich *et al.*, 1990). This suggested that large portions of the genome could be replaced with foreign genetic material. The hepatotropism and persistence (integration) were particularly attractive properties for liver-directed gene transfer. Chang *et al.* (1991) introduced the chloramphenicol acetyltransferase (CAT) gene into duck hepatitis B

virus genome in the place of the polymerase, surface, and pre-surface coding sequences. It was cotransfected with wild-type virus into an avian hepatoma cell line. Culture media containing high titers of the recombinant virus were used to infect primary duckling hepatocytes. Stable CAT gene expression was detected for at least 24 days after transfection (Chang *et al.*, 1991).

5. NON-VIRAL VECTORS

In order to effect expression of the oligonucleotide or polynucleotide sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. As described above, one preferred mechanism for delivery is *via* viral infection where the expression construct is encapsulated in an infectious viral particle.

Once the expression construct has been delivered into the cell the nucleic acid encoding the desired oligonucleotide or polynucleotide sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In certain embodiments of the invention, the expression construct comprising one or more oligonucleotide or polynucleotide sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. Dubensky *et al.* (1984) successfully injected

polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner *in vivo* and express the gene product.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

Selected organs including the liver, skin, and muscle tissue of rats and mice have been bombarded *in vivo* (Yang *et al.*, 1990; Zelenin *et al.*, 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, *i.e. ex vivo* treatment. Again, DNA encoding a particular gene may be delivered *via* this method and still be incorporated by the present invention.

ANTISENSE OLIGONUCLEOTIDES

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic

antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

The targeting of antisense oligonucleotides to mRNA is thus one mechanism to shut down protein synthesis, and, consequently, represents a powerful and targeted therapeutic approach. For example, the synthesis of polygalactauronase and the muscarine type 2 acetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U. S. Patent 5,739,119 and U. S. Patent 5,759,829, each specifically incorporated herein by reference in its entirety). Further, examples of antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal $GABA_A$ receptor and human EGF (Jaskulski *et al.*, 1988; Vasanthakumar and Ahmed, 1989; Peris *et al.*, 1998; U. S. Patent 5,801,154; U. S. Patent 5,789,573; U. S. Patent 5,718,709 and U. S. Patent 5,610,288, each specifically incorporated herein by reference in its entirety). Antisense constructs have also been described that inhibit and can be used to treat a variety of abnormal cellular proliferations, *e.g.* cancer (U. S. Patent 5,747,470; U. S. Patent 5,591,317 and U. S. Patent 5,783,683, each specifically incorporated herein by reference in its entirety).

Therefore, in exemplary embodiments, the invention provides oligonucleotide sequences that comprise all, or a portion of, any sequence that is capable of specifically binding to polynucleotide sequence described herein, or a complement thereof. In one embodiment, the antisense oligonucleotides comprise DNA or derivatives thereof. In another embodiment, the oligonucleotides comprise RNA or derivatives thereof. In a third embodiment, the oligonucleotides are modified DNAs comprising a phosphorothioated modified backbone. In a fourth embodiment, the oligonucleotide sequences comprise peptide nucleic acids or derivatives thereof. In each case, preferred compositions comprise a sequence region that is complementary, and more preferably substantially-complementary, and even more preferably, completely complementary to one or more portions of polynucleotides disclosed herein.

Selection of antisense compositions specific for a given gene sequence is based upon analysis of the chosen target sequence (*i.e.* in these illustrative examples the rat and human sequences) and determination of secondary structure, T_m, binding

energy, relative stability, and antisense compositions were selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell.

Highly preferred target regions of the mRNA, are those which are at or near the AUG translation initiation codon, and those sequences which were substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations were performed using v.4 of the OLIGO primer analysis software (Rychlik, 1997) and the BLASTN 2.0.5 algorithm software (Altschul *et al.,* 1997).

The use of an antisense delivery method employing a short peptide vector, termed MPG (27 residues), is also contemplated. The MPG peptide contains a hydrophobic domain derived from the fusion sequence of HIV gp41 and a hydrophilic domain from the nuclear localization sequence of SV40 T-antigen (Morris *et al.*, 1997). It has been demonstrated that several molecules of the MPG peptide coat the antisense oligonucleotides and can be delivered into cultured mammalian cells in less than 1 hour with relatively high efficiency (90%). Further, the interaction with MPG strongly increases both the stability of the oligonucleotide to nuclease and the ability to cross the plasma membrane (Morris *et al.*, 1997).

RIBOZYMES

Although proteins traditionally have been used for catalysis of nucleic acids, another class of macromolecules has emerged as useful in this endeavor. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim and Cech, 1987; Gerlach *et al.*, 1987; Forster and Symons, 1987). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et al.*, 1981; Michel and Westhof, 1990; Reinhold-Hurek and Shub, 1992). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

Ribozyme catalysis has primarily been observed as part of sequence-specific cleavage/ligation reactions involving nucleic acids (Joyce, 1989; Cech *et al.*, 1981). For example, U. S. Patent No. 5,354,855 (specifically incorporated herein by reference) reports that certain ribozymes can act as endonucleases with a sequence specificity greater than that of known ribonucleases and approaching that of the DNA restriction enzymes. Thus, sequence-specific ribozyme-mediated inhibition of gene expression may be particularly suited to therapeutic applications (Scanlon *et al.*, 1991; Sarver *et al.*, 1990). Recently, it was reported that ribozymes elicited genetic changes in some cells lines to which they were applied; the altered genes included the oncogenes H-*ras*, c-*fos* and genes of HIV. Most of this work involved the modification of a target mRNA, based on a specific mutant codon that is cleaved by a specific ribozyme.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target

RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf *et al.*, 1992). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis δ virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif. Examples of hammerhead motifs are described by Rossi *et al.* (1992). Examples of hairpin motifs are described by Hampel *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz (1989), Hampel *et al.* (1990) and U. S. Patent 5,631,359 (specifically incorporated herein by reference). An example of the hepatitis δ virus motif is described by Perrotta and Been (1992); an example of the RNaseP motif is described by Guerrier-Takada *et al.* (1983); Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990; Saville and Collins, 1991; Collins and Olive, 1993); and an example of the Group I intron is described in (U. S. Patent 4,987,071, specifically incorporated herein by reference). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

In certain embodiments, it may be important to produce enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target, such as one of the sequences disclosed herein. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNA. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) may also be used for exogenous delivery. The simple structure of

these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. Alternatively, catalytic RNA molecules can be expressed within cells from eukaryotic promoters (*e.g.*, Scanlon *et al.*, 1991; Kashani-Sabet *et al.*, 1992; Dropulic *et al.*, 1992; Weerasinghe *et al.*, 1991; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Sarver *et al.*, 1990). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Int. Pat. Appl. Publ. No. WO 93/23569, and Int. Pat. Appl. Publ. No. WO 94/02595, both hereby incorporated by reference; Ohkawa *et al.*, 1992; Taira *et al.*, 1991; and Ventura *et al.*, 1993).

Ribozymes may be added directly, or can be complexed with cationic lipids, lipid complexes, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers.

Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, each specifically incorporated herein by reference) and synthesized to be tested *in vitro* and *in vivo*, as described. Such ribozymes can also be optimized for delivery. While specific examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

Hammerhead or hairpin ribozymes may be individually analyzed by computer folding (Jaeger *et al.*, 1989) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 or so bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Ribozymes of the hammerhead or hairpin motif may be designed to anneal to various sites in the mRNA message, and can be chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described

in Usman *et al.* (1987) and in Scaringe *et al.* (1990) and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. Average stepwise coupling yields are typically >98%. Hairpin ribozymes may be synthesized in two parts and annealed to reconstruct an active ribozyme (Chowrira and Burke, 1992). Ribozymes may be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-o-methyl, 2'-H (for a review see *e.g.*, Usman and Cedergren, 1992). Ribozymes may be purified by gel electrophoresis using general methods or by high pressure liquid chromatography and resuspended in water.

Ribozyme activity can be optimized by altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Int. Pat. Appl. Publ. No. WO 92/07065; Perrault *et al*, 1990; Pieken *et al.*, 1991; Usman and Cedergren, 1992; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ. No. 92110298.4; U. S. Patent 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan *et al.* (Int. Pat. Appl. Publ. No. WO 94/02595) describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination may be locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions

of ribozyme delivery and administration are provided in Int. Pat. Appl. Publ. No. WO 94/02595 and Int. Pat. Appl. Publ. No. WO 93/23569, each specifically incorporated herein by reference.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, *etc.*) present nearby. Prokaryotic RNA polymerase promoters may also be used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990; Gao and Huang, 1993; Lieber *et al.*, 1993; Zhou *et al.*, 1990). Ribozymes expressed from such promoters can function in mammalian cells (*e.g.* Kashani-Saber *et al.*, 1992; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Yu *et al.*, 1993; L'Huillier *et al.*, 1992; Lisziewicz *et al.*, 1993). Such transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral, semliki forest virus, sindbis virus vectors).

Ribozymes may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. They can also be used to assess levels of the target RNA molecule. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These studies will lead to better treatment of the disease progression by affording the possibility of combinational

therapies (*e.g.*, multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes are well known in the art, and include detection of the presence of mRNA associated with an IL-5 related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

PEPTIDE NUCLEIC ACIDS

In certain embodiments, the inventors contemplate the use of peptide nucleic acids (PNAs) in the practice of the methods of the invention. PNA is a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone (Good and Nielsen, 1997). PNA is able to be utilized in a number methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (1997) and is incorporated herein by reference. As such, in certain embodiments, one may prepare PNA sequences that are complementary to one or more portions of the ACE mRNA sequence, and such PNA compositions may be used to regulate, alter, decrease, or reduce the translation of ACE-specific mRNA, and thereby alter the level of ACE activity in a host cell to which such PNA compositions have been administered.

PNAs have 2-aminoethyl-glycine linkages replacing the normal phosphodiester backbone of DNA (Nielsen *et al.*, 1991; Hanvey *et al.*, 1992; Hyrup and Nielsen, 1996; Neilsen, 1996). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; and thirdly, PNA synthesis uses standard Boc (Dueholm *et al.*, 1994) or Fmoc (Thomson *et al.*, 1995) protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used (Christensen *et al.*, 1995).

PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, MA). PNA syntheses by either Boc or Fmoc protocols are straightforward using manual or automated protocols (Norton *et al.*, 1995). The manual protocol lends itself to the production of chemically modified PNAs or the simultaneous synthesis of families of closely related PNAs.

As with peptide synthesis, the success of a particular PNA synthesis will depend on the properties of the chosen sequence. For example, while in theory PNAs can incorporate any combination of nucleotide bases, the presence of adjacent purines can lead to deletions of one or more residues in the product. In expectation of this difficulty, it is suggested that, in producing PNAs with adjacent purines, one should repeat the coupling of residues likely to be added inefficiently. This should be followed by the purification of PNAs by reverse-phase high-pressure liquid chromatography (Norton *et al.*, 1995) providing yields and purity of product similar to those observed during the synthesis of peptides.

Modifications of PNAs for a given application may be accomplished by coupling amino acids during solid-phase synthesis or by attaching compounds that contain a carboxylic acid group to the exposed N-terminal amine. Alternatively, PNAs can be modified after synthesis by coupling to an introduced lysine or cysteine. The ease with which PNAs can be modified facilitates optimization for better solubility or for specific functional requirements. Once synthesized, the identity of PNAs and their derivatives can be confirmed by mass spectrometry. Several studies have made and utilized modifications of PNAs (Norton *et al.*, 1995; Haaima *et al.*, 1996; Stetsenko *et al.*, 1996; Petersen *et al.*, 1995; Ulmann *et al.*, 1996; Koch *et al.*, 1995; Orum *et al.*, 1995; Footer *et al.*, 1996; Griffith *et al.*, 1995; Kremsky *et al.*, 1996; Pardridge *et al.*, 1995; Boffa *et al.*, 1995; Landsdorp *et al.*, 1996; Gambacorti-Passerini *et al.*, 1996; Armitage *et al.*, 1997; Seeger *et al.*, 1997; Ruskowski *et al.*, 1997). U.S. Patent No. 5,700,922 discusses PNA-DNA-PNA chimeric molecules and their uses in diagnostics, modulating protein in organisms, and treatment of conditions susceptible to therapeutics.

In contrast to DNA and RNA, which contain negatively charged linkages, the PNA backbone is neutral. In spite of this dramatic alteration, PNAs

recognize complementary DNA and RNA by Watson-Crick pairing (Egholm *et al.*, 1993), validating the initial modeling by Nielsen *et al.* (1991). PNAs lack 3′ to 5′ polarity and can bind in either parallel or antiparallel fashion, with the antiparallel mode being preferred (Egholm *et al.*, 1993).

Hybridization of DNA oligonucleotides to DNA and RNA is destabilized by electrostatic repulsion between the negatively charged phosphate backbones of the complementary strands. By contrast, the absence of charge repulsion in PNA-DNA or PNA-RNA duplexes increases the melting temperature (T_m) and reduces the dependence of T_m on the concentration of mono- or divalent cations (Nielsen *et al.*, 1991). The enhanced rate and affinity of hybridization are significant because they are responsible for the surprising ability of PNAs to perform strand invasion of complementary sequences within relaxed double-stranded DNA. In addition, the efficient hybridization at inverted repeats suggests that PNAs can recognize secondary structure effectively within double-stranded DNA. Enhanced recognition also occurs with PNAs immobilized on surfaces, and Wang *et al.* have shown that support-bound PNAs can be used to detect hybridization events (Wang *et al.*, 1996).

One might expect that tight binding of PNAs to complementary sequences would also increase binding to similar (but not identical) sequences, reducing the sequence specificity of PNA recognition. As with DNA hybridization, however, selective recognition can be achieved by balancing oligomer length and incubation temperature. Moreover, selective hybridization of PNAs is encouraged by PNA-DNA hybridization being less tolerant of base mismatches than DNA-DNA hybridization. For example, a single mismatch within a 16 bp PNA-DNA duplex can reduce the T_m by up to 15°C (Egholm *et al.*, 1993). This high level of discrimination has allowed the development of several PNA-based strategies for the analysis of point mutations (Wang *et al.*, 1996; Carlsson *et al.*, 1996; Thiede *et al.*, 1996; Webb and Hurskainen, 1996; Perry-O'Keefe *et al.*, 1996).

High-affinity binding provides clear advantages for molecular recognition and the development of new applications for PNAs. For example, 11-13 nucleotide PNAs inhibit the activity of telomerase, a ribonucleo-protein that extends

telomere ends using an essential RNA template, while the analogous DNA oligomers do not (Norton *et al.*, 1996).

Neutral PNAs are more hydrophobic than analogous DNA oligomers, and this can lead to difficulty solubilizing them at neutral pH, especially if the PNAs have a high purine content or if they have the potential to form secondary structures. Their solubility can be enhanced by attaching one or more positive charges to the PNA termini (Nielsen *et al.*, 1991).

Findings by Allfrey and colleagues suggest that strand invasion will occur spontaneously at sequences within chromosomal DNA (Boffa *et al.*, 1995; Boffa *et al.*, 1996). These studies targeted PNAs to triplet repeats of the nucleotides CAG and used this recognition to purify transcriptionally active DNA (Boffa *et al.*, 1995) and to inhibit transcription (Boffa *et al.*, 1996). This result suggests that if PNAs can be delivered within cells then they will have the potential to be general sequence-specific regulators of gene expression. Studies and reviews concerning the use of PNAs as antisense and anti-gene agents include Nielsen *et al.* (1993b), Hanvey *et al.* (1992), and Good and Nielsen (1997). Koppelhus *et al.* (1997) have used PNAs to inhibit HIV-1 inverse transcription, showing that PNAs may be used for antiviral therapies.

Methods of characterizing the antisense binding properties of PNAs are discussed in Rose (1993) and Jensen *et al.* (1997). Rose uses capillary gel electrophoresis to determine binding of PNAs to their complementary oligonucleotide, measuring the relative binding kinetics and stoichiometry. Similar types of measurements were made by Jensen *et al.* using BIAcore™ technology.

Other applications of PNAs include use in DNA strand invasion (Nielsen *et al.*, 1991), antisense inhibition (Hanvey *et al.*, 1992), mutational analysis (Orum *et al.*, 1993), enhancers of transcription (Mollegaard *et al.*, 1994), nucleic acid purification (Orum *et al.*, 1995), isolation of transcriptionally active genes (Boffa *et al.*, 1995), blocking of transcription factor binding (Vickers *et al.*, 1995), genome cleavage (Veselkov *et al.*, 1996), biosensors (Wang *et al.*, 1996), *in situ* hybridization (Thisted *et al.*, 1996), and in a alternative to Southern blotting (Perry-O'Keefe, 1996).

POLYPEPTIDE COMPOSITIONS

The present invention, in other aspects, provides polypeptide compositions. Generally, a polypeptide of the invention will be an isolated polypeptide (or an epitope, variant, or active fragment thereof) derived from a mammalian species. Preferably, the polypeptide is encoded by a polynucleotide sequence disclosed herein or a sequence which hybridizes under moderately stringent conditions to a polynucleotide sequence disclosed herein. Alternatively, the polypeptide may be defined as a polypeptide which comprises a contiguous amino acid sequence from an amino acid sequence disclosed herein, or which polypeptide comprises an entire amino acid sequence disclosed herein.

In the present invention, a polypeptide composition is also understood to comprise one or more polypeptides that are immunologically reactive with antibodies generated against a polypeptide of the invention, particularly a polypeptide having the amino acid sequence disclosed in SEQ ID NO:126, or to active fragments, or to variants or biological functional equivalents thereof.

Likewise, a polypeptide composition of the present invention is understood to comprise one or more polypeptides that are capable of eliciting antibodies that are immunologically reactive with one or more polypeptides encoded by one or more contiguous nucleic acid sequences contained in SEQ ID NO:1-125, or to active fragments, or to variants thereof, or to one or more nucleic acid sequences which hybridize to one or more of these sequences under conditions of moderate to high stringency. Particularly illustrative polypeptides include the amino acid sequence disclosed in SEQ ID NO:126.

As used herein, an active fragment of a polypeptide includes a whole or a portion of a polypeptide which is modified by conventional techniques, *e.g.*, mutagenesis, or by addition, deletion, or substitution, but which active fragment exhibits substantially the same structure function, antigenicity, etc., as a polypeptide as described herein.

In certain illustrative embodiments, the polypeptides of the invention will comprise at least an immunogenic portion of a breast tumor protein or a variant thereof, as described herein. As noted above, a "breast tumor protein" is a protein that

is expressed by breast tumor cells. Proteins that are breast tumor proteins also react detectably within an immunoassay (such as an ELISA) with antisera from a patient with breast cancer. Polypeptides as described herein may be of any length. Additional sequences derived from the native protein and/or heterologous sequences may be present, and such sequences may (but need not) possess further immunogenic or antigenic properties.

An "immunogenic portion," as used herein is a portion of a protein that is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of a breast tumor protein or a variant thereof. Certain preferred immunogenic portions include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other preferred immunogenic portions may contain a small N- and/or C-terminal deletion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.*, they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native breast tumor protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be

immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I-labeled Protein A.

As noted above, a composition may comprise a variant of a native breast tumor protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native breast tumor protein in one or more substitutions, deletions, additions and/or insertions, such that the immunogenicity of the polypeptide is not substantially diminished. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

Polypeptide variants encompassed by the present invention include those exhibiting at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity (determined as described above) to the polypeptides disclosed herein.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and

alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide.

As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means,

using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc.* *85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide

folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene 40*:39-46, 1985; Murphy *et al.*, *Proc. Natl. Acad. Sci. USA 83*:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided. Such proteins comprise a polypeptide as described herein together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see*, for example, Stoute *et al. New Engl. J. Med.*, *336*:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.*, the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred

embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

BINDING AGENTS

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a breast tumor protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a breast tumor protein if it reacts at a detectable level (within, for example, an ELISA) with a breast tumor protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a cancer, such as breast cancer, using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a breast tumor protein will generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the cancer. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.*, blood, sera, sputum, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an

antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* *6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture

supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ^{90}Y, ^{123}I, ^{125}I, ^{131}I, ^{186}Re, ^{188}Re, ^{211}At, and ^{212}Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-

containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter *et al.*), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn *et al.*), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler *et al.*).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be

coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato *et al.*), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih *et al.*). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison *et al.* discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

T CELLS

Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for a breast tumor protein. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO

92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with a breast tumor polypeptide, polynucleotide encoding a breast tumor polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, a breast tumor polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a breast tumor polypeptide if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen *et al.*, *Cancer Res.* *54*:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (*e.g.*, by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a breast tumor polypeptide (100 ng/ml - 100 μg/ml, preferably 200 ng/ml - 25 μg/ml) for 3 - 7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (*e.g.*, TNF or IFN-γ) is indicative of T cell activation (*see* Coligan *et al.*, Current Protocols in Immunology, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a breast tumor polypeptide, polynucleotide or polypeptide-expressing APC may be $CD4^+$ and/or $CD8^+$. Breast tumor protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are

derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, $CD4^+$ or $CD8^+$ T cells that proliferate in response to a breast tumor polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a breast tumor polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a breast tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of a breast tumor protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

PHARMACEUTICAL COMPOSITIONS

In additional embodiments, the present invention concerns formulation of one or more of the polynucleotide, polypeptide, T-cell and/or antibody compositions disclosed herein in pharmaceutically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

It will also be understood that, if desired, the nucleic acid segment, RNA, DNA or PNA compositions that express a polypeptide as disclosed herein may be administered in combination with other agents as well, such as, *e.g.*, other proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable

dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation.

1. ORAL DELIVERY

In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz *et al.*, 1997; Hwang *et al.*, 1998; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

2. INJECTABLE DELIVERY

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally as described in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of

storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U. S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event,

determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

3. NASAL DELIVERY

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described *e.g.,* in U. S. Patent 5,756,353 and U. S. Patent 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.,* 1998) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroetheylene support matrix is described in U. S. Patent 5,780,045 (specifically incorporated herein by reference in its entirety).

4. LIPOSOME-, NANOCAPSULE-, AND MICROPARTICLE-MEDIATED DELIVERY

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into suitable host cells. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the nucleic acids or constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the

art (see for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon and Papahadjopoulos, 1988; Allen and Choun, 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, 1990; Muller *et al.*, 1990). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs (Heath and Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta and Puglisi, 1996), radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi *et al.*, 1990b), viruses (Faller and Baltimore, 1984), transcription factors and allosteric effectors (Nicolau and Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trails examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b; Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori and Fukatsu, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 µm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, *i.e.* in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur *et al.* (1977; 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for h or days, depending on their composition, and half lives in the blood range from min to several h. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the

antibody and its drug contents to specific antigenic receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be are easily made, as described (Couvreur *et al.*, 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684, specifically incorporated herein by reference in its entirety).

VACCINES

In certain preferred embodiments of the present invention, vaccines are provided. The vaccines will generally comprise one or more pharmaceutical compositions, such as those discussed above, in combination with an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (*e.g.*, polylactic galactide) and liposomes (into which the compound is incorporated; *see e.g.*, Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic

portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

Illustrative vaccines may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems 15*:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch *et al.*, *Proc. Natl. Acad. Sci. USA 86*:317-321, 1989; Flexner *et al.*, *Ann. N.Y. Acad. Sci. 569*:86-103, 1989; Flexner *et al.*, *Vaccine 8*:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques 6*:616-627, 1988; Rosenfeld *et al.*, *Science 252*:431-434, 1991; Kolls *et al.*, *Proc. Natl. Acad. Sci. USA 91*:215-219, 1994; Kass-Eisler *et al.*, *Proc. Natl. Acad. Sci. USA 90*:11498-11502, 1993; Guzman *et al.*, *Circulation 88*:2838-2848, 1993; and Guzman *et al.*, *Cir. Res. 73*:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that a vaccine may comprise both a polynucleotide and a polypeptide component. Such vaccines may provide for an enhanced immune response.

It will be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (*e.g.*, salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (*e.g.*, sodium, potassium, lithium, ammonium, calcium and magnesium salts).

While any suitable carrier known to those of ordinary skill in the art may be employed in the vaccine compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344 and 5,942,252. One may also employ a carrier comprising the particulate-protein complexes described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

Such compositions may also comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a

lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (*e.g.*, IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (*e.g.*, IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; *see* US Patent

Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science 273*:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties.

Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (*see, e.g.*, Coombes *et al.*, *Vaccine 14*:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a

polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.*, a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature 392*:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman and Levy, *Ann. Rev. Med. 50*:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up,

process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel *et al.*, *Nature Med. 4*:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.*, CD54 and CD11) and costimulatory molecules (*e.g.*, CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a breast tumor protein (or portion or other variant thereof) such that the breast tumor polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a composition or vaccine comprising such

transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi *et al.*, *Immunology and cell Biology* *75*:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the breast tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (*e.g.*, vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (*e.g.*, a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Vaccines and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a vaccine or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

CANCER THERAPY

In further aspects of the present invention, the compositions described herein may be used for immunotherapy of cancer, such as breast cancer. Within such methods, pharmaceutical compositions and vaccines are typically administered to a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with cancer. Accordingly, the above pharmaceutical compositions and vaccines may be used to prevent the development of a cancer or to treat a patient afflicted with a cancer. A cancer may be diagnosed using criteria generally accepted in the art, including the presence of a malignant tumor.

Pharmaceutical compositions and vaccines may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. Administration may be by any suitable method, including administration by intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents (such as polypeptides and polynucleotides as provided herein).

Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T cells as discussed above, T lymphocytes (such as $CD8^+$ cytotoxic T lymphocytes and $CD4^+$ T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages) expressing a polypeptide provided herein. T cell receptors and antibody receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other vectors or effector cells for adoptive immunotherapy. The polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (as described above and in U.S. Patent No. 4,918,164) for passive immunotherapy.

Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro*, as described herein. Culture conditions for expanding single antigen-specific effector cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture conditions typically use intermittent stimulation with antigen, often in the presence of cytokines (such as IL-2) and non-dividing feeder cells. As noted above,

immunoreactive polypeptides as provided herein may be used to rapidly expand antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast and/or B cells, may be pulsed with immunoreactive polypeptides or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*. Studies have shown that cultured effector cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (*see*, for example, Cheever *et al.*, *Immunological Reviews 157*:177, 1997).

Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.

Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50% above the basal (*i.e.*, untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such vaccines should also be capable of causing an immune response that

leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 25 µg to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a breast tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

CANCER DETECTION AND DIAGNOSIS

In general, a cancer may be detected in a patient based on the presence of one or more breast tumor proteins and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, sputum urine and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer such as breast cancer. In addition, such proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence or absence of a cancer. In general, a breast tumor sequence should be present at a level that is at least three fold higher in tumor tissue than in normal tissue

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. *See*, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory,

1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In a preferred embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length breast tumor proteins and portions thereof to which the binding agent binds, as described above.

The solid support may be any material known to those of ordinary skill in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is

preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 µg, and preferably about 100 ng to about 1 µg, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see*, *e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact

time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with breast cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of a cancer, such as breast cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value for the detection of a cancer is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three

standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett *et al.*, *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above.

Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1μg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to those of ordinary skill in the art that the above protocols may be readily modified to use breast tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such breast tumor protein specific antibodies may correlate with the presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with a breast tumor protein in a biological sample. Within certain methods, a biological sample comprising $CD4^+$ and/or $CD8^+$ T cells isolated from a patient is incubated with a breast tumor polypeptide, a polynucleotide encoding such a polypeptide and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected. Suitable biological samples include, but are not limited to, isolated T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with polypeptide (*e.g.*, 5 - 25 μg/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of breast tumor polypeptide to serve as a control. For $CD4^+$ T cells, activation is preferably detected by evaluating proliferation of the T cells. For $CD8^+$ T cells, activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a breast tumor protein in a biological sample. For

example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify a portion of a breast tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for (*i.e.*, hybridizes to) a polynucleotide encoding the breast tumor protein. The amplified cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes that specifically hybridize to a polynucleotide encoding a breast tumor protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the tumor protein in a biological sample.

To permit hybridization under assay conditions, oligonucleotide primers and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding a breast tumor protein that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length. In a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule having a sequence recited in SEQ ID NO:1-___. Techniques for both PCR based assays and hybridization assays are well known in the art (*see*, for example, Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, *51*:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989).

One preferred assay employs RT-PCR, in which PCR is applied in conjunction with reverse transcription. Typically, RNA is extracted from a biological sample, such as biopsy tissue, and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification may be performed on biological samples taken from a test patient and from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-

fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered positive.

In another embodiment, the compositions described herein may be used as markers for the progression of cancer. In this embodiment, assays as described above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) or polynucleotide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the level of polypeptide or polynucleotide detected increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide or polynucleotide either remains constant or decreases with time.

Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound binding agent may then be detected directly or indirectly via a reporter group. Such binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

As noted above, to improve sensitivity, multiple breast tumor protein markers may be assayed within a given sample. It will be apparent that binding agents specific for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that results in optimal sensitivity. In addition, or alternatively, assays for tumor proteins provided herein may be combined with assays for other known tumor antigens.

DIAGNOSTIC KITS

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a breast

tumor protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a breast tumor protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a breast tumor protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a breast tumor protein.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

IDENTIFICATION OF BREAST TUMOR PROTEIN cDNAS

This Example illustrates the identification of cDNA molecules encoding breast tumor proteins.

Potential breast-specific genes present in the GenBank human EST database were identified by electronic subtraction (similar to that described by Vasmatizis et al., *Proc. Natl. Acad. Sci. USA 95*:300-304, 1998). The sequences of EST clones (26,074) derived from various human breast cDNA libraries were obtained from the GenBank public human EST database. Each breast EST sequence was used as a query sequence in a BLASTN (National Center for Biotechnology Information) search against the human EST database. All matches considered identical (BLAST score > 40, length of matching sequence >100 base pairs, density of identical matches over this region > 80%) were grouped (aligned) together in a cluster. Clusters containing more than 200 ESTs were discarded since they probably represented repetitive elements or highly expressed genes such as those for ribosomal proteins. If two or more clusters shared common ESTs, those clusters were grouped together into a "supercluster," resulting in 9,974 breast superclusters.

Records for the 479 human cDNA libraries represented in the GenBank release were downloaded to create a database of these cDNA library records. These 479 cDNA libraries were grouped into three groups, Plus (normal breast and breast tumor libraries, and breast cell lines, in which expression was desired), Minus (libraries from other normal adult tissues, in which expression was not desirable), and Other (fetal tissue, infant tissue, ovary, pregnant uterus, male-specific tissues, non-breast tumors and cell lines other than breast cell lines, in which expression was considered to be irrelevant). A summary of these library groups is presented in Table I.

TABLE I

BREAST cDNA LIBRARIES

Library	# of Libraries
Plus	11
Normal	6
Tumor	4
Cell lines	1
Minus	171
Other	297

Each supercluster was analyzed in terms of the ESTs within the supercluster. The tissue source of each EST clone was noted and used to classify the superclusters into four groups: Type 1 - EST clones derived from the Plus group libraries only; no EST clones derived from Minus or Other group libraries; Type 2 - EST clones derived from the Plus and Other group libraries only; no EST clones derived from the Minus group; Type 3 - EST clones derived from the Plus, Minus and Other group libraries, but the number of EST clones derived from the Plus group is higher than from either the Minus or Other groups; and Type 4 - EST clones derived from Plus, Minus and Other group libraries, but the number of clones derived from the Plus group is higher than the number from the Minus group. For each type, subcategories were generated for clusters containing one or two clones. Types 1a, 2a, 3a and 4a represent Type 1, 2, 3 and 4 clusters where the number of clones in the Plus group is 2. Types 1b, 2b, 3b and 4b represent Type 1, 2, 3 and 4 clusters where the number of clones in the Plus group is 1. This analysis identified 3230 breast clusters. From these clusters, 2501 EST clones were ordered from Research Genetics, Inc. (Huntsville, AL), and were received as frozen glycerol stocks in 96-well plates. A summary of the clusters generated and clones ordered is shown in Table II.

TABLE II

BREAST CLUSTER SUMMARY

Type	# of Superclusters	# of ESTs Ordered
1	50	49
1a (2/0/0)	130	130
1b (1/0/0)	65	65
2	167	162
2a (2/0/n)	312	321
2a (1/0/n)	1875	1752
3	56	22
3a	32	0
4	333	0
4a	210	0
Total	3230	2501

The EST clone inserts were PCR-amplified for Synteni microarray analysis using amino-linked PCR primers. When more than one PCR product was obtained for a particular clone, that clone was not used for expression analysis. In total, 1896 clones from the electronic subtraction method were analyzed by microarray analysis to identify electronic subtraction breast clones that had high tumor vs. normal tissue mRNA. Such screens were performed using a Synteni (Palo Alto, CA) microarray, according to the manufacturer's instructions (and essentially as described by Schena et al., *Proc. Natl. Acad. Sci. USA 93*:10614-10619, 1996 and Heller et al., *Proc. Natl. Acad. Sci. USA 94*:2150-2155, 1997). Within these analyses, the clones were arrayed on the chip, which was then probed with fluorescent probes generated from normal and tumor breast cDNA, as well as various other normal tissues. The slides were scanned and the fluorescence intensity was measured.

Clones with an expression ratio greater than 2 (*i.e.*, the level in breast tumor cDNA was at least twice the level in normal breast cDNA) were identified as

breast tumor-specific sequences (Table III). The sequences of these clones are provided in SEQ ID NOs:1-124.

TABLE III

BREAST-TUMOR SPECIFIC CLONES

SEQ ID NO.	Sequence Designation
1	19702
2	19703
3	B1003C
4	B1002C
5	19708
6	19709
7	19710
8	19711
9	B1006C
10	B1007C
11	19714
12	19715
13	19716
14	19717
15	19718
16	19719
17	19720
18	19721
19	19997
20	19998
21	19999
22	20000
23	20001
24	20002
25	20005
26	20006
27	20007
28	20008
29	20009
30	20010
31	20011
32	20012
33	20013
34	20014

35	20079
36	20080
37	20081
38	20082
39	20083
40	20085
41	20086
42	20087
43	20088
44	20089
45	20090
46	20091
47	20092
48	20093
49	20115
50	20116
51	20118
52	20119
53	20120
54	20121
55	20122
56	20123
57	20124
58	20125
59	20360
60	20361
61	20362
62	22180
63	22181
64	22182
65	22183
66	22185
67	22186
68	22188
69	22189
70	22190
71	22191
72	22192
73	22193
74	22194
75	22196
76	22197
77	22198
78	22199
79	22200
80	22201

81	22202
82	22204
83	22206
84	22207
85	22208
86	22209
87	22210
88	22211
89	22212
90	22213
91	22214
92	22215
93	22216
94	22217
95	22218
96	22219
97	22220
98	22221
99	22222
100	22223
101	22224
102	22225
103	22226
104	22227
105	22228
106	22229
107	22230
108	22231
109	22232
110	22233
111	22234
112	22235
113	22236
114	22237
115	22238
116	22239
117	22240
118	22241
119	22242
120	22243
121	22244
122	22245
123	22334
124	22335

The B1002C sequence (SEQ ID NO:4; 517 bp) was used in a BlastN search of the GenBank Human EST database to identify overlapping sequences that extended further toward the 5' end of the corresponding gene. Two human EST clones were identified in this manner and were purchased from Genome Systems (St. Louis, MO) and sequenced. The resulting sequence information gave rise to a partial predicted open reading frame of 214 amino acids, which is 88% identical over 180 amino acids to the mouse "iroquois homeobox protein 3" (GenBank protein accession #Y15001). The extended B1002C sequence, along with the predicted open reading frame, are provided herein as SEQ ID NOs:125 and 126, respectively. The alignment between B1002C and the mouse iroquois homeobox protein 3 is presented in Figure 1.

EXAMPLE 2

SYNTHESIS OF POLYPEPTIDES

Polypeptides may be synthesized on a Perkin Elmer/Applied Biosystems Division 430A peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed:

1. An isolated polypeptide, comprising at least an immunogenic portion of a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125;

(b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, under moderately stringent conditions; and

(c) complements of sequences of (a) or (b).

2. An isolated polypeptide according to claim 1, wherein the polypeptide comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing polynucleotide sequences.

3. An isolated polypeptide comprising a sequence recited in SEQ ID NO:126.

4. An isolated polynucleotide encoding at least 15 amino acid residues of a breast tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ

ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing sequences.

5. An isolated polynucleotide encoding a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing sequences.

6. An isolated polynucleotide, comprising a sequence recited in any one of SEQ ID NOs: 1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125.

7. An isolated polynucleotide, comprising a sequence that hybridizes to a sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, under moderately stringent conditions.

8. An isolated polynucleotide complementary to a polynucleotide according to any one of claims 4-7.

9. An expression vector, comprising a polynucleotide according to any one of claims claim 4-8.

10. A host cell transformed or transfected with an expression vector according to claim 9.

11. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a breast tumor protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing polynucleotide sequences.

12. A fusion protein, comprising at least one polypeptide according to claim 1.

13. A fusion protein according to claim 12, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.

14. A fusion protein according to claim 12, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.

15. A fusion protein according to claim 12, wherein the fusion protein comprises an affinity tag.

16. An isolated polynucleotide encoding a fusion protein according to claim 12.

17. A pharmaceutical composition, comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:

(a) a polypeptide according to claim 1;
(b) a polynucleotide according to claim 4;
(c) an antibody according to claim 11;
(d) a fusion protein according to claim 12; and
(e) a polynucleotide according to claim 16.

18. A vaccine comprising an immunostimulant and at least one component selected from the group consisting of:

(a) a polypeptide according to claim 1;

(b) a polynucleotide according to claim 4;

(c) an antibody according to claim 11;

(d) a fusion protein according to claim 12; and

(e) a polynucleotide according to claim 16.

19. A vaccine according to claim 18, wherein the immunostimulant is an adjuvant.

20. A vaccine according to any claim 18, wherein the immunostimulant induces a predominantly Type I response.

21. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a pharmaceutical composition according to claim 17.

22. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a vaccine according to claim 18.

23. A pharmaceutical composition comprising an antigen-presenting cell that expresses a polypeptide according to claim 1, in combination with a pharmaceutically acceptable carrier or excipient.

24. A pharmaceutical composition according to claim 23, wherein the antigen presenting cell is a dendritic cell or a macrophage.

25. A vaccine comprising an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NOs:1-125;

(b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-125 under moderately stringent conditions; and

(c) complements of sequences of (i) or (ii);

in combination with an immunostimulant.

26. A vaccine according to claim 25, wherein the immunostimulant is an adjuvant.

27. A vaccine according to claim 25, wherein the immunostimulant induces a predominantly Type I response.

28. A vaccine according to claim 25, wherein the antigen-presenting cell is a dendritic cell.

29. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NOs:1-125;

(b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-125 under moderately stringent conditions; and

(c) complements of sequences of (i) or (ii)encoded by a polynucleotide recited in any one of SEQ ID NOs:1-125;

and thereby inhibiting the development of a cancer in the patient.

30. A method according to claim 29, wherein the antigen-presenting cell is a dendritic cell.

31. A method according to any one of claims 21, 22 and 29, wherein the cancer is breast cancer.

32. A method for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(i) polynucleotides recited in any one of SEQ ID NOs:1-125; and

(ii) complements of the foregoing polynucleotides;

wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the antigen from the sample.

33. A method according to claim 32, wherein the biological sample is blood or a fraction thereof.

34. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated according to the method of claim 32.

35. A method for stimulating and/or expanding T cells specific for a breast tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

(a) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(i) sequences recited in SEQ ID NOs:1-125;

(ii) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-125 under moderately stringent conditions; and

(iii) complements of sequences of (i) or (ii);

(b) polynucleotides encoding a polypeptide of (a); and

(c) antigen presenting cells that express a polypeptide of (a);

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

36. An isolated T cell population, comprising T cells prepared according to the method of claim 35.

37. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population according to claim 36.

38. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating $CD4^{+}$ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(1) sequences recited in SEQ ID NOs:1-125;

(2) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-125 under moderately stringent conditions; and

(3) complements of sequences of (1) or (2);

(ii) polynucleotides encoding a polypeptide of (i); and

(iii) antigen presenting cells that expresses a polypeptide of (i);

such that T cells proliferate; and

(b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient.

39. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating $CD4^+$ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(1) sequences recited in SEQ ID NOs:1-125;

(2) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-125 under moderately stringent conditions; and

(3) complements of sequences of (1) or (2);

(ii) polynucleotides encoding a polypeptide of (i); and

(iii) antigen presenting cells that express a polypeptide of (i);

such that T cells proliferate;

(b) cloning at least one proliferated cell to provide cloned T cells; and

(c) administering to the patient an effective amount of the cloned T cells, and thereby inhibiting the development of a cancer in the patient.

40. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with a binding agent that binds to a breast tumor protein, wherein the tumor protein comprises an amino acid

sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-125 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent; and

(c) comparing the amount of polypeptide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

41. A method according to claim 40, wherein the binding agent is an antibody.

42. A method according to claim 43, wherein the antibody is a monoclonal antibody.

43. A method according to claim 40, wherein the cancer is breast cancer.

44. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-125 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polypeptide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

45. A method according to claim 44, wherein the binding agent is an antibody.

46. A method according to claim 45, wherein the antibody is a monoclonal antibody.

47. A method according to claim 44, wherein the cancer is a breast cancer.

48. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NO:1-125 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and

(c) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

49. A method according to claim 48, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

50. A method according to claim 48, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

51. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide

sequence recited in any one of SEQ ID NO:1-125 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polynucleotide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

52. A method according to claim 51, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

53. A method according to claim 51, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

54. A diagnostic kit, comprising:

(a) one or more antibodies according to claim 11; and

(b) a detection reagent comprising a reporter group.

55. A kit according to claim 54, wherein the antibodies are immobilized on a solid support.

56. A kit according to claim 54, wherein the detection reagent comprises an anti-immunoglobulin, protein G, protein A or lectin.

57. A kit according to claim 54, wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

58. An oligonucleotide comprising 10 to 40 contiguous nucleotides that hybridize under moderately stringent conditions to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing polynucleotides.

59. A oligonucleotide according to claim 58, wherein the oligonucleotide comprises 10-40 contiguous nucleotides recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125.

60. A diagnostic kit, comprising:

(a) an oligonucleotide according to claim 59; and

(b) a diagnostic reagent for use in a polymerase chain reaction or hybridization assay.

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1   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  B1002Corf.PRO
1   H S F P Q L G Y Q Y I R P L Y P P E R F G A A G G G G G G S S A G G R S G F G A G A S E L A A S G S  iroquois.PRO

1   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  B1002Corf.PRO
51  L S N V L S S V Y G A P Y A A A A A A A A A A A Q G Y G A F L P Y A T E L P I F F Q L G A Q Y E L K D  iroquois.PRO

1   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  B1002Corf.PRO
101 S P G V Q H P A T A A A F P H P H P A F Y P Y G Q Y Q F G D F S R F K H A T R E S T S T L K A W L N  iroquois.PRO

1   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  B1002Corf.PRO
151 E H R K N P Y P T K G E K I H L A I I T K H T L T Q V S T W F A H A R R R L K K E N K H T W A P R S  iroquois.PRO

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201 R T D E E G N A Y G S E R E E E D E E E D E E E S K R E L E H E E E E L A G R G G G H G G R G A G R  iroquois.PRO

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1   R R R D E E I D L E N L D S A A A G S E L T L A G A A H R H G D F G L G P I S D C K T S D S D D S S  iroquois.PRO

11  F E R G A G R G S S G G R E E G A R R R E E G L A P R R H V H Q S L H P C A P A P A P A S A L Q K P  B1002Corf.PRO
301 E G L E D R P L S V L S L A P P P P P V A R A P A S P P S P P S S L D P C A P A P A P S S A L Q K P  iroquois.PRO

61  K I W S L A R T A T S P D N P R R S P P G A G G S P P G A A V A P S A L Q L S P A A A A A A A - - H  B1002Corf.PRO
351 K I W S L A E T A T S P D N P R R S P P G A G G S P P G A A V A P P T L Q L S P A A A A A A A A A H  iroquois.PRO

109 P L V S A P L G K F P A W T N R P P P Q P P P G P R P H P L S L L G S A P P H L L G L P G A A G H P  B1002Corf.PRO
401 R L V S A P L G K F P A W T N R P P P G P P A G P R P H P L S H L G S A P Q H L L G L P G A A G H P  iroquois.PRO

159 A A A A A - F A R P A R P R G G T D R C S A L E V E K K L L P T A P Q P V P H P P Q H H L H A A L V  B1002Corf.PRO
451 A A A A A A Y A R P A E P E S G T D R C S A L E V E K K L L K T A F Q P V P H H P Q H H L H A A L V  iroquois.PRO

200 L S A L S S S                                                                                          B1002Corf.PRO
501 L S A L S S S                                                                                          iroquois.PRO
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FIG 1

SEQUENCE LISTING

<110> Corixa Corporation
Mitcham, Jennifer L.
Jiang, Yuqiu

<120> COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER

<130> 210121.482PC

<140> PCT
<141> 2000-06-23

<160> 127

<170> FastSEQ for Windows Version 3.0

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<213> Homo sapien

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ccattatact tctcacaatt cagtttctat gagtttgatc acctgatttt tttaacaaaa    480
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gagtagcttc acaaagatag gagataaccg agaaactgtt tttattgcat cttcctgtgt 240
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<212> DNA
<213> Homo sapien

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<211> 499
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<213> Homo sapien

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caaaaaaaaa aaaaaaaaa 499

<210> 6
<211> 88
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(88)
<223> n = A,T,C or G

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<210> 7
<211> 777
<212> DNA
<213> Homo sapien

<400> 7
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cacgtgcacc gtgacccaca cagacctgcc ctcgccactg aagcagacca tctcccggcc 240
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gtccctggtc atgtccgaca cagctggcac ctgctactga ccctgctggc ctgcccacag 660
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<211> 996
<212> DNA
<213> Homo sapien

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aagttggatg gcaggtagag ttcaaccagt tcgtgactgt tgtagcgaat gaagttaaaa 780
aaatgtcttt ctgatgttgt gttgtcattt tcatttttgc attttttgt ttgcatatta 840
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cattgttaac gggccttatt ctgatctcac ctgtcgcgta gctctaatat tcacataaac 960
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<210> 9

<211> 811
<212> DNA
<213> Homo sapien

<400> 9
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cagccaacac cgagtgtacg tggaccatca acccaccccc caagcgccgc atcctgatcg 180
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aagacatagt tcgagatggc aggctctatg catctgagaa ccatcaggaa atacttaagg 480
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gttctgctat agggttggtg ggacagagct gtcttccttc tgcatgtcag cacagtcggg 720
tattgctgcc tcccgtatca gtgactcatt agagttcaat ttttatagat aattcagata 780
ttttggtaaa ttgaaaaaaa aaaaaaaaaa a 811

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<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(542)
<223> n = A,T,C or G

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tatctaaggg tattctatgt gctaatataa aatattttta acacttgaga acagggatct 480
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<210> 11
<211> 508
<212> DNA
<213> Homo sapien

<400> 11
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catgggggag gctgccacag gtcacactcc ttagccggat cccctcctga agaaaagcat 180
ctgagctgac cagcgagact gcagtggggc agacactatt aaatttgctg gcacagctct 240
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gactactctg ctggcgacac ggccacta 508

<210> 12
<211> 836
<212> DNA
<213> Homo sapien

<400> 12
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aggccaggga tgctgctcaa cctcctacca tgtacaggac gtctccccat tacaactacc 180
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tcagcttgtt gagcttctaa gtttctttcc cttcattcta ccctgcaagc caagttctgt 480
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gaaaagaata ctttgtttcc agccccccttc ccacactytt catgtgttaa ccactgcctt 720
cctggacctt ggagccacgg tgactgtatt acatgttgtt atagaaaact gattttagag 780
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<211> 530
<212> DNA
<213> Homo sapien

<400> 13
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agtgtgtgtg ggtgaaatgg agatttggaa ttgaactctc tgcctgtaaa tgttccccaa 480
ataattgttg tgtgtatgat acgtgtataa taaaagtatt cttgttagaa 530

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<211> 809
<212> DNA
<213> Homo sapien

<220>
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<222> (1)...(809)
<223> n = A,T,C or G

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<211> 866
<212> DNA
<213> Homo sapien

<400> 15
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<210> 16
<211> 500
<212> DNA
<213> Homo sapien

<400> 16
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cataaagaaa gttttatttg tattttacta ctgtttggtc aattttgata tataactggt 180
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cttcttgact aaaactggtc 500

<210> 17
<211> 732
<212> DNA
<213> Homo sapien

<400> 17

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cctgcagtga gccaccgcgg caggcacgtt gactgtttta ctgacatcac tcaaaagcta 480
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cctactgatc ccattcgttg cttctattgt aaatatttgt catttgtatt tattatctct 660
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<210> 18
<211> 103
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(103)
<223> n = A,T,C or G

<400> 18
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<210> 19
<211> 586
<212> DNA
<213> Homo sapien

<400> 19
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cagccggaga acaactacaa gaccacgcct cccgtgctgg actccgacgg ctccttcttc 300
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<210> 20
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<212> DNA
<213> Homo sapien

<400> 20
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gcaggcacac aacagaggca gttccagatt tcaactgctc atcagatggc gggaagatga 360
agacagatgg tgcagccaca gttcgtttga tctccagctt ggtcccctgg ccaaaagtgt 420
acggaggtga gctgtcgacg cggccgcgaa tttagtagta gtaggcggcc gctctagagg 480
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<210> 21
<211> 625
<212> DNA
<213> Homo sapien

<400> 21
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acactgtacc atctcagtcc cactctgcct gtaacttaaa aaacagcccc taccccccaga 180
gggtctgcga gttaatacct tgagaatagt ctacagtttt tcatagtttg tctgagctag 240
aaaacttgta cctgtaaaac aaaggacagc attgaggact gaaacttgtc tytttttttg 300
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tctagtgtct agaattacaa agaataaaat gaaatcaaag atttctcgct agtaaaatga 420
aatgttagga acagtattaa aatataggtc ctaccccaac gacacttaca cagagcccag 480
tacagtacct attattaaca ggacgcatag cttaaggagg aaccacatca aatcttcagc 540
cagacatatc tagcctcaga agtgcaaaaa aaaaaaaaag cccccaaacg aagacacccc 600
actgagtagg gtgcatgccg tgagt 625

<210> 22
<211> 576
<212> DNA
<213> Homo sapien

<400> 22
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tgggctggca gtagcccgag atgatgggct cttctctggg gatcccaact ggttccctaa 180
gaaatccaag gagaatcctc ggaacttctc ggataaccag ctgcaagagg gcaagaacgt 240
gatcgggtta cagatgggca ccaaccgcgg ggcgtctcag gcaggcatga ctggctacgg 300
gatgccacgc cagatcctct gatcccaccc caggccttgc ccctgccctc ccacgaatgg 360
ttaatatata tgtagatata tattttagca gtgacattcc cagagagccc cagagctctc 420
aagctccttt ctgtcagggt gggggttca gcctgtcctg tcacctctga ggtgcctgct 480
ggcatcctct cccccatgct tactaataca ttcccttccc catagccatc aaaactggac 540
caactggcct cttcctttcc cctgggacca aaattt 576

<210> 23
<211> 416
<212> DNA
<213> Homo sapien

<400> 23
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ccggggactc tcttccagcc tccgaccgcc ctccgatttc ctctccgctt gcaacctccg 120
ggaccatctt ctcggccatc tcctgcttct gggacctgcc agcaccgttt ttgtggttag 180
ctccttcttg ccaaccaacc atgagctccc agattcgtca gaattattcc accgacgtgg 240
aggcagccgt caacagcctg gtcaatttgt acctgcaggc ctcctacacc tacctctctc 300
tgggcttcta tttcggaccg cgatgatgtg gctctggaag gcgtgagcca cttcttccgc 360

gaactggccg aggagaagcg cgagggctac gagcgtctcc tgaagatgca aaacca 416

<210> 24
<211> 1037
<212> DNA
<213> Homo sapien

<400> 24
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ttcccagcac aggctctgtg gagatggctg tcgcagtgtt cctggtcatc tgcattgtgg 180
tggtggtagc catcttgggt tactgcttct tcaagaacca gagaaaggac ttccacggac 240
accaccacca cccaccaccc acccctgcca gctccactgt ctccactacc gaggacacgg 300
agcacctggt ctataaccac accaccccggc ccctctgagc ctgggtctca ccggctctca 360
cctggccctg cttcctgctt gccaaggcag aggcctgggc tgggaaaaac tttggaacca 420
gactcttgcc tgtttcccag gcccactgtg cctcagagac cagggctcca gcccctcttg 480
gagaagtctc agctaagctc acgtcctgag aaagctcaaa ggtttggaag gagcagaaaa 540
cccttgggcc agaagtacca gactagatgg acctgcctgc ataggagttt ggaggaagtt 600
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ggcctagacc agcagacaga gccaggagag gstcagctgc attccgcagc ccccaccccc 960
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aaaaaaaaaa aaaaaaa 1037

<210> 25
<211> 1144
<212> DNA
<213> Homo sapien

<400> 25
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gaattcaagt gtgaccctca tgaggcaacg tgttacgatg atgggaagac ataccacgta 180
ggagaacagt ggcagaagga atatctcggt gccatttgct cctgcacatg ctttggaggc 240
cagcggggct ggcgctgtga caactgccgc agacctgggg gtgaacccag tcccgaaggc 300
actactggcc agtcctacaa ccagtattct cagagatacc atcagagaac aaacactaat 360
gttaattgcc caattgagtg cttcatgcct ttagatgtac aggctgacag agaagattcc 420
cgagagtaaa tcatctttcc aatccagagg aacaagcatg tctctctgcc aagatccatc 480
taaactggag tgatgttagc agacccagct tagagttctt ctttctttct taagcccttt 540
gctctggagg aagttctcca gcttcagctc aactcacagc ttctccaagc atcaccctgg 600
gagtttcctg agggttttct cataaatgag ggctgcacat tgcctgttct gcttcgaagt 660
attcaatacc gctcagtatt ttaaatgaag tgattctaag atttggtttg ggatcaatag 720
gaaagcatat gcagccaacc aagatgcaaa tgttttgaaa tgatatgacc aaaattttaa 780
gtaggaaagt cacccaaaca cttctgcttt cacttaagtg tytggcccgc aatactgtag 840
gaacaagcat gatcttgtta ctgtgatatt ttaaatatcc mcagtactca ctttttccaa 900
atgatcctag taattgccta gaaatatctt tctcttacct gttatttatc aatttttccc 960
agtattttta tacggaaaaa attgtattga aaacmcttag tatgcagttg ataagaggaa 1020
tttggtataa ttatggtggg tgaaaatttt ttatactgta tgtgccaaag ctttaaaact 1080
gtggaaagac aactgtttta ataaaagatt tacattcccc aacttgaaaa aaaaaaaaaa 1140
aaaa 1144

<210> 26

<211> 488
<212> DNA
<213> Homo sapien

<400> 26
gcatttttgt ctgtgctccc tgatcttcag gtcaccacca tgaagttctt agcagtcctg 60
gtactcttgg gagtttccat ctttytggtc tctgcccaga atccgacaac agctgctcca 120
gctgacacgt atccagctac tggtcctgct gatgatgaag cccctgatgc tgaaaccact 180
gctgctgcaa ccactgcgac cactgctgct cctaccactg caaccaccgc tgcttctacc 240
actgctcgta aagacattcc agttttaccc aaatgggttg gggatctccc gaatggtaga 300
gtgtgtccct gagatggaat cagcttgagt cttctgcaat tggtcacaac tattcatgct 360
tcctgtgatt tcatccaact acttaccttg cctacgatat ccccttttatc tctaatcagt 420
ttattttytt tcaaataaaa aataactatg agcaacataa aaaaaaaaaa aaaaaaaaaa 480
aaaaaaa 488

<210> 27
<211> 764
<212> DNA
<213> Homo sapien

<400> 27
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attcagggca tgtctctccc ccaggcccca tcttcataca cttccgctaa gatgcccaca 120
attcatcccc cttcatgtgc agcttgaaaa accctgacat tgttgctccc tgccgggcat 180
acctgccatt gccactgccc atctctgtga tgaggaattc aacaccagat ctctggcagc 240
tcttgaggga ttggccagtg gccattctca ggggtgctgt ctagttttcc cagagccccg 300
ccaaggtcct ggccaccgtg gcagagcggg gaggttcccc cactctaaag cctactcctt 360
ctctctctag cctcttcagg aggtggtggt agcagcagca atttccacat caatgtagaa 420
gagtcagtgg atggacaggt ggtttcttcc cacaagagag aaatytaagt gtctattgca 480
ggagaaacgt cccttgccac tccccactct catcaggcca agtggaggac tggccagagg 540
gcctgcacat gcaaactcca gtccctgcct tcagagagct gaaaagggtc cctcggtctt 600
ttatttcagg gctttgcatg cgctctattc cccctctgcc tctccccmcc ttytttggag 660
caaggagatg cagctgtatt gtgtaacaag ctcatttgta cagtgtctgt tcatgtaata 720
aagaattact tttccttttg caaaaaaaaa aaaaaaaaaa aaaa 764

<210> 28
<211> 802
<212> DNA
<213> Homo sapien

<400> 28
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ccagggaaag cccctaacct cctgatctat gctgcatcca ctttgcaaag tggggtccca 120
tcaaggttca stggcagtga atctgggaca gatttcactc tcaccatcag cagtctgcaa 180
cctgaagatt ttgcaactta ctactgycaa cagagttaca ctaccccatt cactttcrgm 240
cctgggacca aagtggatgt caaacgaact gtggctgcac catctgtctt catcttcccg 300
ccatctgatg agcagwtgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 360
tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc 420
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg 480
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgsgaagt cacccatcag 540
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgttagag ggagaagtgc 600
ccccacctgc tcctcagttc cagcctgacc ccctcccatc ctttggccty tgaccctttt 660
tccacagggg acctacccct attgcggtcc tccagctcat ctttcacctc accccccctcc 720
tcctccttgg ctttaattat gctaatgttg gaggagaatg aataaataaa gtgaatcttt 780
gcaaaaaaaa aaaaaaaaaa aa 802

<210> 29
<211> 620
<212> DNA
<213> Homo sapien

<400> 29
ctcgagccag aattcggtcg aggagaactg gtataggagc tccaaggaca agaaacacgt 60
ctggctagga gaaactatca atgctggcag ccagtttgaa tataatgtag aaggagtgac 120
ttccaaggaa atggctaccc aacttgcctt catgcgcctg ctggccaact atgcctctca 180
gaacatcacc taccactgca agaacagcat tgcatacatg gatgaggaga ctggcaacct 240
gaaaaaggct gtcattctac agggctctaa tgatgttgaa cttgttgctg agggcaacag 300
caggttcact tacactgttc ttgtagatgg ctgctctaaa aagacaaatg aatggggaaa 360
gacaatcatt gaatacaaaa caaataagcc atcacgcctg cccttccttg atattgcacc 420
tttggacatc ggtggtgctg accaggaatt ctttgtggac attggcccag tctgtttcaa 480
ataaatgaac tcaatctaaa ttaaaaaaga aagaaatttg aaaaaacttt ctctttgcca 540
tttcttcttc ttcttttta actgaaagct gaatccttcc atttcttctg cacatctact 600
tgcttaaatt gtgggcaaaa 620

<210> 30
<211> 644
<212> DNA
<213> Homo sapien

<400> 30
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aaccaccmgc cmatttaaca taaaacactt gkcaagctga gtagactgkt ttcttatgkg 120
aaccacaaaa tattttctct gaaatctaca cttagtttaa aaacagagat gggatttgc 180
atattagctt gaaaataagt atatgatgat gatattaggk gcccactagc acctagtttt 240
tacagctttg cattgkcacc ccatcactgc cagggaccca gccccaggca tacacagatg 300
aaaggacagt ttcaccttct tggcaaaaac cttcagaaca attgtcaaca tactctcaaa 360
tgtctttccc actcagaaat gaggagcaag gtgtatgact ttagattcaa gaagtatatg 420
gggctaaata tctttaaaag tataactctg gacaatgtac ttagggacct actacttact 480
caaaataggg tagtagcatt aataactaaa tagggcgtga gtcatgagaa agattccctg 540
catctcttgc caagtattaa cactagtttt actaataagc aacggggtag ataactttga 600
agattgcatc aacttcaaca caaaaaaatg tgaatatccc tcgt 644

<210> 31
<211> 674
<212> DNA
<213> Homo sapien

<400> 31
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tcaagagtaa tccacacgtg agaaactaga caatgtacat tcatgtgttc tcttgaaagg 120
aaagggagag ctgtaagctt cactctgtcc tacaccggag aaaagcagga ataactttac 180
cgtggaaata atgtttagct tttatcagag aaaattgtcc ttctagagca tagagtccca 240
aaactcaatt ctggttttcc cctgtttttt tttttttttt ttttcccaac atatgaactg 300
cagcatatca ctttttcttt ttgtgcctca ggttcctcag ctgtaaaatt gaaaaatata 360
tgtattaata atattattaa taataataat ggtaatgtag tacttgtttg taaagcactt 420
tgagatcctt ggttgaaagg caccatagga gtgccaagta ttattatgtg gccaaggggg 480
ttatttaaac tgtcagttcc caaaggccag gaaaggttgg ggtcattttt cttaaagacg 540
agctgtaaat atcaactagg cagccaatag tgttgactat gaagatgcaa aactattact 600
aggmtgataa aatcatagtt tcttaatggc taccaataag gcaaatatca caataataaa 660
caccaaattc cgta 674

<210> 32
<211> 713
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(713)
<223> n = A,T,C or G

<400> 32
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gccagaattc ggtcgaggac agctataaaa gaaagctgtt atttactgta gttgtagatg    60
tattcactac akaatcctac atttttcagc aggccacagt ccanccaaat cctataatat   120
ccttgaaaag aaactattaa aaaggataga catttctgat gtaagtaaaa cccccaagca   180
acttaatatc catctgtcag tcatcaactt ttcccctaga tttttttttt aactagttct   240
gaaagtgtca agaatagctt ccctttttcac cctacctagg ttccctcctt gctggcataa   300
aggccaagca gtgaaatgaa ccttgggtta gagttatggt aggcaaaaag aaaaggagaa   360
tttaggaaat aaatatgcta taaacaaggt acagtacagt gagagttaac aatcctagga   420
atcctgcctg tcacaagctc ccagttccct gtcattttat cttcatgatt agaactgatc   480
ttccatttaa ctattacaga aagcagtaac tattgcaact atctaatagt tcacacaaaa   540
gaattaaaag cttaaaaata atttttagga aacacaatat tcaaaatcta aacacactga   600
taaattatta agattaagat tatwtatgtg ataaatgaaa tctcctacca atccatccag   660
cctttaccag ggaagaaaag caattatttc atttcagata gaaatacaaa aaa          713
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<210> 33
<211> 698
<212> DNA
<213> Homo sapien

<400> 33
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tcgagggctc caccgaaaga gtcactggaa ctggaggacc cgtcttctgg gctgggtgtg    60
accaagcagg atctgggccc agtccccatg tgagagcagc agaggcggtc ttcaacatcc   120
tgccagcccc acacagctac agctttcttg ctcccttcag cccccagccc ctccccccatc  180
tcccaccctg tacctcatcc catgagaccc tggtgcctgg ctctttcgtc acccttggac   240
aagacaaacc aagtcggaac agcagataac aatgcagcaa ggccctgctg cccaatctcc   300
atctgtcaac aggggcgtga ggtcccagga agtggccaaa agctagacag atccccgttc   360
ctgacatcac agcagcctcc aacacaaggc tccaagacct aggttcatgg acgagatggg   420
aaggcacagg gagaagggat aaccctacac ccagacccca ggctggacat gctgactgtc   480
ctctcccctc cagcctttgg ccttggcttt tctagcctat ttacctgcag gctgagccac   540
tctcttccct ttccccagca tcactcccca aggaagagcc aatgttttcc acccataatc   600
ctttctgccg acccctagtt ccctctgctc agccaagcta gwtatcagct ttcagggcca   660
tggttcacat tagaataaaa ggtagtaatt agaaaaaa                           698
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<210> 34
<211> 605
<212> DNA
<213> Homo sapien

<400> 34
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aaggtgccat cattcttgag gaggaagtag cgtggccgcc aggtcttgat gtactcccct    60
cgtttgtgca gccaaccctc cttcacaata gccacgtcgc tcatggtgcc cgaggctccc   120
gcgacgctca cgcgctcctc tcaggctggc gctccccgag cccagctggc ctggccacag   180
cctctgatgc accagctgac aggctgcctc ctccaggcag cccctttgac ttctttgacc   240
caggctggct cggccttccc taagcccctg gtgacagatg gccccgtttg ctctccctgt   300
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ccatggtgtt cctacccatg gatcctggga cagggcacag ggctcctccc tgctccccag      360
actaggaaag caaagaaatt caaacatgag gaagacagga ccaggatgca ggccactggc      420
gcaaacggga gtccagagcc ctccagcgca agcccaaaaa cctcctggga gaaaccccag      480
gcccctccta aaccacagcg cccctgccgg tctgaatctg gttcattcat ttggccaaca      540
tgtaccgggc gtctcaggtt ttgccaggcc cagtgctggg cgctggagac aaagagcctc      600
gtrcc                                                                  605

      <210> 35
      <211> 753
      <212> DNA
      <213> Homo sapien

      <220>
      <221> misc_feature
      <222> (1)...(753)
      <223> n = A,T,C or G

      <400> 35
atggagacgg ctctgcagca gtacactcta gaaccatcag aaaaaccttt tgttctcaag       60
tctgtgcccc tggccacggc gcccatggca gagcagagaa cagaaagtac cccccatcaca     120
scagtcaaac agcctgagaa agtggcagct accaggcagg agatcttcca ggagcagttg      180
gcagcagtgc cagagttccg cggtcttggg cccctcttca agtcctcgcc tgagcccgtg      240
gccctcaccg agtcagagac ggagtatgtc atccgctgca ccaaacacac cttcaccaac      300
cacatggttt ttcagtttga ctgcacaaac acactcaatg accagacctt ggagaatgtc      360
acagtgcaga tggagcccac tgaggcctat gaggtgctct gttacgtgcc tgcccggagc      420
ctgccctaca accagcccgg gacctgctac acactggtgg cactgcccaa agaagacccc      480
acagctgtgg cctgcacatt cagctgcatg atgaagttca ctgtcaagga ctgtgatccc      540
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gttactgtag ctgatcacat tcaaaaggtc atgaaactga acttcgaagc agcctgggat      660
gangtagggg atgaatttga gaaggaggaa acgttcacct tgtctaccat caagacactt      720
gaagaggctg ggggnaatat tgggaagtct tgg                                   753

      <210> 36
      <211> 433
      <212> DNA
      <213> Homo sapien

      <400> 36
ggggcttgca atcatttgaa ttgttctgtt tcacaataaa ggagattcac tgggttctgc       60
attttcagga ttcaatagaa ctgcttcatt aaaaaaataa tccttagcaa gcattcgaat      120
cctaactgct ttgatgcact tgccctcggg cacctgtcat ttccaatatg gtaggtgtca      180
aagtcaaaag tatttactgg gagaaaaaag agaggagtgg ttgtagaagt ctccctaaat      240
cagacatgtc aagcaatcag ccaacgtggt gtatttctca ttcaatattt tagtgtgaat      300
tgagacactg agataaagac atcgtgcaga gataaatggg gatacagtta aatgtagcaa      360
ctcttgagtt catttttttcc cactgtagca aaattaatgc tttctcttta ttgaaataaa     420
ttgctcattc ctc                                                         433

      <210> 37
      <211> 601
      <212> DNA
      <213> Homo sapien

      <220>
      <221> misc_feature
      <222> (1)...(601)
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<223> n = A,T,C or G

<400> 37
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agttcgaaaa gggccccttt gggaagaact gcagcgcggc gtgtccgggc ctgcagctgt 120
cgaacaaccc cgtgaagggc aggacctgca aggagaggga ctcagagggc tgctgggtgg 180
cctacacgct ggagcagcag gacgggatgg accgctacct catctatgtg gatgagagcc 240
gagagtgtgt ggcaggcccc aacatcgccg ccatcgtcgg gggcaccgtg gcaggcatcg 300
tgctgatcgg cattctcctg ctggtcatct ggaaggctct gatccacctg agcgacctcc 360
gggagtacag gcgctttgag aaggagaagc tcaagtccca gtggaacaat gataatcccc 420
ttttcaagag cgccaccacg acggtcatga accccaagtt tgctgagagt taggagcact 480
tggtgaaaac aanggccgtc aggacccacc atgtctgccc atacgcggcc ganacatggc 540
ttggcacact nttgangatg taccatttac caaaatcnan tttttcccct tnaatnanct 600
g 601

<210> 38
<211> 713
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(713)
<223> n = A,T,C or G

<400> 38
gcttccgcct attgattctg ctcatttata caataatgag gagcaggttg gactggccat 60
ccgaagcaag attgcagatg gcaktgtgaa gagagaagac atattctaca cttcaaagct 120
ttggtgcaat tcccatcgac cagagttggt ccgaccagcc ttggaaaggt cactgaaaaa 180
tcttcaattg gattatgttg acctctacct tattcatttt ccagtgtctg taaagccagg 240
tgaggaagtg atcccaaaag atgaaaatgg aaaaatacta tttgacacag tggatctctg 300
tgccacgtgg gaggccgtgg agaagtgtaa agatgcagga ttggccaagt ccatcggggt 360
gtccaacttc aaccgcaggc agctggagat gatcctcaac aagccagggc tcaagtacaa 420
gcctgtctgc aaccaggtgg aatgtcatcc ttacttcaac cagagaaaac tgctggattt 480
ctgcaagtca aaagacattg ttctggttgc ctatagtgct ctgggatccc accgagaaga 540
accatgggtg gacccgaact ccccggtgct cttggaggac ccagtccttt gtgccttggc 600
aaaaaagcac aagcgaaccc cagccctgat tgccctgcgc taccactaca gcgtggggtt 660
gtggtcctgg ccaagagcta caatgagcag cgcatcanac agaacgtgca ggg 713

<210> 39
<211> 451
<212> DNA
<213> Homo sapien

<400> 39
gaaagaactg actgaaacgt ttgagatgaa gaaagttctc ctcctgatca cagccatctt 60
ggcagtggct gttggtttcc cagtctctca agaccaggaa cgagaaaaaa gaagtatcag 120
tcgacagcga tgaattagct tcagggtttt ttgtgttccc ttacccatat ccatttcgcc 180
cacttccacc aattccattt ccaagatttc catggtttag acgtaatttt cctattccaa 240
tacctgaatc tgcccctaca actccccttc ctagcgaaaa gtaaacaaga aggaaaagtc 300
acgataaacc tggtcacctg aaattgaaat tgagccactt ccttgaagaa tcaaaattcc 360
tgttaataaa agaaaaacaa atgtaattga aatagcacac agcattctct agtcaatatc 420
tttagtgatc ttctttaata aacttgaaag c 451

<210> 40

<211> 778
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(778)
<223> n = A,T,C or G

<400> 40
accgcatcct agccgccgac tcacacaagg caggtgggtg aggaaatcca gagttgccat 60
ggagaaaatt ccagtgtcag cattcttgct ccttgtggcc ctctcctaca ctctggccag 120
asataccaca gtcaaacctg gagccaaaaa ggacacaaag gactctcgac ccaaactgcc 180
ccagacccctc tccagaggtt ggggtgacca actcatctgg actcagacat atgaagaagc 240
tctatataaa tccaagacaa gcaacaaacc cttgatgatt attcatcact tggatgagtg 300
cccacacagt caagctttaa agaaagtgtt tgctgaaaat aaagaaatcc agaaattggc 360
agagcagttt gtcctcctca atctggttta tgaaacaact gacaaacacc tttctcctga 420
tggccagtat gtccccagga ttatgtttgt tgacccatct ctgacagtta gagccgatat 480
cactggaaga tattcaaatc gtctctatgc ttacgaacct gcagatcagc tctgttgctt 540
gacaacatga agaaagctct caagttgctg aagactgaat tgtaaagaaa aaaaatctcc 600
aagcccttct gtctgtcagg ccttgagact tgaaaccaga anaagtgtga naaagactgg 660
ctagtggggg aagcattagt ggaacacact ggattanggt tatgggtttn aatggttacc 720
aacaacctat ttttttttaa naaaaaaacc aaagttttta aaaaaaattt ngggtttt 778

<210> 41
<211> 696
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(696)
<223> n = A,T,C or G

<400> 41
agtgatgccg gcagatcatc tggtttcagc gggacnacgt atcttgcatg tggtctggca 60
tggaccncag ggctgtgggg acttggggta cagtaatcaa gtaatcccct tttccagaat 120
gcattaaccc actccctttg acctcacgat ggggcaggtc cccaagtgtg caagctcagt 180
attcatgatg gtgggggatg gagtgtcttc cgaggttctt gggggaaaaa atattgtagc 240
atatttaagg gaggcaatga accctctccc ccncntcttc cctgcccaaa tctgtctcct 300
agaatcttat gtgctgtgaa taataggcct tcwctgcccc tccagttttt atagacstga 360
ggttccagtg tctcctggta actggaacct ytcctgaggg ggaatcctgg tgctcaaatt 420
accctccaaa agcaagtagc caaagccgtt gccaaacccc acccataaat caatgggccc 480
tttatttatg mcgactttat ttattctaat atgattttat agtatttata tatattgggt 540
cgtctgcttc ccttgtattt ttmttccttt ttttgtaata ttgaaaasgg sgatataatt 600
attataagta gactataata tatttagtaa tatatattat taccttaaaa gtctattttt 660
gtgttttggg catttttaaa taaacartct gagtgt 696

<210> 42
<211> 509
<212> DNA
<213> Homo sapien

<400> 42
ctcaggtgga aaaggaggga gctactctca ggctgcgtgc agcgacagtg cccagggctc 60

tgatgtgtct ctcacagctt gaaaagcctg agacagctgt cttgtgaggg actgagatgc 120
acgatttctt cacgcctccc ctttgtgact tcaagagcct ctggcatctc tttctgcaaa 180
ggcacctgaa tgtgtctgcg tccctgttag cataatgtga ggaggtggag agacagccca 240
cccttgtgtc cactgtgacc cctgttccca tgctgacctg tgtttcctcc ccagtcatct 300
ttcttgttcc acaagaggtg ggctggatg tctccatctc tgtctcaact ttacgtgcac 360
tgagctgcaa cttcttactt ccctactgaa aataagaatc tgaatataaa tttgttttct 420
caaatatttg ctatgagagg ttgatggatt aattaaataa gtcaattcct ggaatttgga 480
gagagcaaat aaagacctga gaaccttcc 509

<210> 43
<211> 388
<212> DNA
<213> Homo sapien

<400> 43
gcaggatttc ttcactcctc ccctttgtga cttcaagagc ctctggcatc tctttctgca 60
aaggcacctg aatgtgtctg cgtccctgtt agcctaatgt gaggaggtgg agagacagcc 120
cacccttgtg tccactgtga cccctgttcc catgctgacc tgtgtttcct ccccagtcat 180
ctttcttgtt ccagagaggt ggggctggat gtctccatct ctgtctcaac tttatgtgca 240
ctgagctgca acttcttact tccctactga aaataagaat ctgaatatac atttgttttc 300
tcaaawattt gctatgagag gttgatggat taattaaata agtcaattcc tggaatgtga 360
gagagcaaat aaagacctga gaaccttc 388

<210> 44
<211> 565
<212> DNA
<213> Homo sapien

<400> 44
ggaaaagagg gttcctctcc ttgtgwgtgt ctcttccccc cacccctaat tcttctgctc 60
tgtttgggaa gacgtggagg aaaaggtgac ttctgccccc accgctctta cccccaccaa 120
ctctcgcgtg ttggagagaa ggggccctcc cagcacaaag ttgcattcct ccccccctaat 180
ttattctaat ttattaactt tgacccaccc tttctgagcc tgcagccttc ccgtgtggcc 240
tgagggctgt cgagtgagct gccccagccc ctcccagccc ttgcccagcc tgggggagtg 300
gggaaggctt gggcatggcc ccgttggagg ttgatttgct gttttgtttc ttgtctttgt 360
gttctgtggt acttgctgag agaaaagaaa agtgagccaa gcagaaggag gtgggaaaac 420
ggacccaaac cccagtgtgc cctgccccga tgcctttcct ttagtggtgg gaaaccctta 480
tctygcaaag tgaatgtgtc cccttcccca cccthtagtg tatttcacag aaaacaaaac 540
ctcccaataa aacggttgaa gcctg 565

<210> 45
<211> 661
<212> DNA
<213> Homo sapien

<400> 45
gttgaggaga tgggatgtcc cagatgatag ggctcctggg atttcagacc caataccagc 60
aggactccag tcacctctac cccakttctc caggacacag cgctcccaac tctgagtgac 120
gtcccacctc tggtccttgc agcacaacca acgtgggaat cacaccctcc agacctccca 180
cagctccacc ccagactggg cgccggccct gcctccattt cagctgtgac aacctcagag 240
ccgtgttggc ccaagcatga caaggacgta tgaaaacttc cagtacttgg agaataaggt 300
gaaagtccag ggtttaaaa atgggccact tcctctccag tccctcctgc agcgtctccg 360
ctctgggccc tgccatctcc tgctgtccct gggcctcggc ctgctgctgc tggtcatcat 420
ctgtgtggtt ggattccaaa attccaaatt tcagagggac ctggtgaccc tgagaacaga 480
ttttagcaac ttcacctcaa acactgtggc ggagatccag gcactgactt cccagggcag 540

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cagcttggaa gaaacgatag catctctgaa agctgaggtg gagggtttca agcaggaacg     600
gcaggcagtt cattctgaaa tgctcctgcg agtccagcag ctggtgcaag acctgaagaa     660
a                                                                     661

      <210> 46
      <211> 453
      <212> DNA
      <213> Homo sapien

      <400> 46
tgcctctgtt gtgtgcctgc tgaataactt ctatcccaga gaggccaaag tacagtggaa      60
ggtggataac gccctccaat cgggtatctc ccaggagagt gtcacagagc aggacagcaa     120
ggacagcacc tacagcctca gcagcaccct gacgctgagc aaagcagact acgagaaaca     180
caaagtctac gcctgcgaag tcacccatca gggcctgagc tcgcccgtca caaagagctt     240
caacagggga gagtgttaga gggagaagtg cccccacctg ctcctcagtt ccagcctgac     300
cccctcccat cctttggcct ctgacccttt ttccacaggg gacctacccc tattgcggtc     360
ctccagctca tctttcacct caccccccctc ctcctccttg gctttaatta tgctaatgtt     420
ggaggagaat gaataaataa agtgaatctt tgc                                  453

      <210> 47
      <211> 687
      <212> DNA
      <213> Homo sapien

      <220>
      <221> misc_feature
      <222> (1)...(687)
      <223> n = A,T,C or G

      <400> 47
cttcggctaa aatgcagaaa tgcatgctgt cagcgttggt atttcacatt caatggagct      60
gaatgttsag gacctcttcc cattgaagct ataatttatt tggaccaagg aagccctgaa     120
atgaattcaa caattaatat tcatcgcact tcttctgtgg aaggactttg tgaaggaatt     180
ggtgctggat tagtggatgt tgctatctgg gttggcactt gttcagatta cccaaaagga     240
gatgcttcta ctggatggaa ttcagtttct cgcatcatta ttgaagaact accaaaataa     300
atgctttaat tttcatttgc tacctctttt tttattatgc cttggaatgg ttcacttaaa     360
tgacatttta aataagttta tgtatacatc tgaatgaaaa gcaaagctaa atatgtttac     420
agaccaaagt gtgatttcac actgttttta aatctagcat tattcatttt gcttcaatca     480
aaagtggttt caatattttt tttagttggt tagaatactt tcttcatagt cacattctct     540
caacctataa tttggaatat tgttgtggct tttgtttttt ctcttagtat agcattttta     600
aaaaaatata aaagctacca atctttgtca atttgtaaat gttaaanaat ttttttttata     660
tctggtnaaa taaaaaatta ttttccc                                         687

      <210> 48
      <211> 944
      <212> DNA
      <213> Homo sapien

      <400> 48
ggcacgagga gcatggacat gagggtcccc gctcagctcc tggggctcct gctactctgg      60
ctcccgaggtg ccagatgtga catccagatg acccagtctc catcttccct gtctgcttct    120
gtaggagaca gagtcaccat cacttgccgg gcaagtcaga acattaacaa cttcttaatt    180
tggtatcagc aaaaaccagg gagacccccct cagctcctga tctatcttac atctaatttg    240
caaagtgggg tcccatctag gttcagtggc agtggatctt ggacagattt cactctcacc    300
atcaccggtc tgcagcctga agattttgca acttactact gtcaacagag tcacagtacc    360
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ccgtacacct tcggccaggg gaccaagctg gagatcaaac gaactgtggc tgcaccatct 420
gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 480
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 540
caatcgggta actcccagga gagtgtcaca gagcaggaca gcaaggacag cacctacagc 600
ctcagcagca ccctgacgct gagcaaagca gactacgaga aacacaaact ctacgcctgc 660
gaagtcaccc atcagggcct gagctctccc gtcacaaaga gcttcaacag gggagagtgt 720
tagagggaga agtgccccca cctgctcctc agttccagcc tgaccccctc ccatcctttg 780
gcctctgacc ctttttccac aggggaccta cccctattgc ggtcctccag ctcatctttc 840
acctcacccc cctcctcctc cttggcttta attatgctaa tgttggagga gaatgaataa 900
ataaagtgaa tctttgcacc tgtaaaaaaa aaaaaaaaaa aaaa 944

<210> 49
<211> 449
<212> DNA
<213> Homo sapien

<400> 49
ggcacgaggg cttcgaggaa ccagccctga tacctgtgac cctctctgta cgctaccctc 60
ctgaagtgtc catctccggc tatgatgaca actggtacct cggccgtact gatgccaccc 120
tgagctgtga cgtccgcagc aacccagagc ccacgggcta tgactggagc acgacctcag 180
gcaccttccc gacctccgca gtggcccagg gctcccagct ggtcatccac gcagtggaca 240
gtctgttcaa taccaccttc gtctgcacag tcaccaatgc cgtgggcatg ggccgcgctg 300
agcaggtcat ctttgtccga gaaacccccа gggcctcgcc ccgagatgtg ggcccgctgg 360
tgtggggggc cgtggggggg acactgctgg tgctgctgct tctggctggg gggtccttgg 420
ccttcatcct gctgagggtg aggaggagg 449

<210> 50
<211> 703
<212> DNA
<213> Homo sapien

<400> 50
ggcacgaggg tgagcccatg ccccacccaa ccctgccagc tgagcaaagg acagtcttac 60
agcgtcaatg tcaccttcac cagcaatatt cagtctaaaa gcagcaaggc cgtggtgcat 120
ggcatcctga tgggcgtccc agttcccttt ctcattcctg agcctgatgg ttgtaagagt 180
ggaattaact gccctatcca aaaagacaag acctatagct acctgaataa actaccagtg 240
aaaagcgaat atccctctat aaaactggtg gtggagtggc aacttcagga tgacaaaaac 300
caaagtctct tctgctggga aatcccagta cagatcgttt ctcatctcta agtgcctcat 360
tgagttcggt gcatctggcc aatgagtctg ctgagactct tgacagcacc tccagctctg 420
ctgcttcaac aacagtgact tgctctccaa tggtatccag tgattcgttg aagaggaggt 480
gctctgtagc agaaactgag ctccgggtgg ctggttctca gtggttgtct catgtctctt 540
tttctgtctt aggtggtttc attaaatgca gcacttggtt agcagatgtt taattttttt 600
tttaacaaca ttaacttgtg gcctctttct acacctggaa atttactctt gaataaataa 660
aaactcgttt gtcttgtcaa aaaaaaaaaa aaaaaaaaaa aaa 703

<210> 51
<211> 963
<212> DNA
<213> Homo sapien

<400> 51
caatgaggct ccctgctcag ctcctggggc tgctaatgct ctgggtctct ggatccagtg 60
gggatattgt gatgactcag tctccactct cccagcccgt cacccctgga gagccggcct 120
ccatctcctg caagtctagt cagagcctcc tacatagtaa tggatacagt tatttggatt 180
ggtacctgca gaagccagga cagtctccac agctcctgat ctatttggct tctaagcggg 240

cctccggggt ccctgacagg ttcagtggca gtggatcagg cacagatttt acactgagaa 300
tcagtagagt ggaggctgag gatgttggct tttattactg catgcaaact ctacagaccc 360
cgctcacttt cggcggaggg accaaggtgg agatcaaacg aactgtggct gcaccatctg 420
tcttcatctt cccgccatct gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc 480
tgctgaataa cttctatccc agagaggcca aagtacagtg gaaggtggat aacgccctcc 540
aatcgggtaa ctcccaggag agtgtcacag agcaggacag caaggacagc acctacagcc 600
tcagcagcac cctgacgctg agcaaagcag actacgagaa acacaaactc tacgcctgcg 660
aagtcaccca tcagggcctg agctcgcccg tcacaaagag cttcaacagg ggagagtgtt 720
agagggagaa gtgccccccc ctgctcctca gttccagcct gacccccctcc catcctttgg 780
cctctgaccc tttttccaca ggggacctac ccctattggg gtcctccagb tcwtttttcc 840
cctccccccc ctcctcctcc ttggbtttaa ttatgytaak gttggrggar aatgaataaa 900
taaagtgawt ttttgmaaaa aaaaaaaaaa aaaaaaaaaa aaaaagaaaa aaaaaaaaaa 960
aaa 963

<210> 52
<211> 628
<212> DNA
<213> Homo sapien

<400> 52
cctttctctc tcctcatttc ggtgcatgtc ctttctgcag ctgcctttca gcacaggtgg 60
ttccactggg ggcagctaac gctgagtgac aaggatggga agccacaggt gcattttact 120
caagtcttct ctagtcaatg aggggcaccc agtgcttctg ggcaggctgg ggtggtggtc 180
ccctaggtat cagcctctct tactgtactc tccgggaatg ttaacctttc tattttcagc 240
ctgtgccacc tgtctaggca agctggcttc cccattggcc cctgtgggtc cacagcagcg 300
tggctgcccc ccagggccac cgcttctttc ttgatcctct ttccttaaca gtgacttggg 360
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tggccctctc cagggtgttt tccactagtc actactgtct tctccttgta gctaatcaat 540
caatattctt cccttgcctg tgggcagtgg agagtgctgc tgggtgtacg ctgcacctgc 600
ccactgagtt ggggaaagag gataatca 628

<210> 53
<211> 598
<212> DNA
<213> Homo sapien

<400> 53
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tcccctaacc cccagtcctc actgtcctgt tttcttaggc actgagggat ctgagtaggt 120
ctgggatgga caggaaaggg caaagtaggg cgtgtggttt ccctgcccct gtccggaccg 180
ccgatcccag gtgcgtgtgt ctctgtctct cctagcccct ytytcacaca tcacattccc 240
atggtggcct caaraaaggc ccggaagcgc caggctggag ataacagcct yttgcccgtc 300
ggccctgcgt cggccctggg gtaccatgtg gccacaactg ctgtggcccc ctgtccccaa 360
racacttccc cttgtctccc tggttgccty tcttgcccct tgtcctgaag cccagcgaca 420
caraaggggg tggggcgggt ytatggggar aaagggagcg aggtcaragg agggcatggg 480
ttggcagggt gggcgtttgg ggccctytat gctggctttt caccccarag gacacaggca 540
gcttccaaaa wawatttaty ttcttcacgg gaaaaaaaaa aaaaaaaaaa aaaaaaa 598

<210> 54
<211> 592
<212> DNA
<213> Homo sapien

<400> 54

gtgtttacac agtaagatca agagtcaggc cctggaattt ccagaaccag cccgacatag 60
ttgaggactt gaaggacctg atcacccygt atgctggaca agaaccccga gtcgaggatc 120
gtggtgccgk aaatcaagat mctggtraag accatratac gtaaacgctc ctttgggaac 180
ccattcgagg gcasccggmg ggaggaacgc tcactgtcag ygcctggaaa cttgctcacr 240
aagcaaggca gygaagacaa cctccagggc wccgacccgc cccccgtggg ggaggaggaa 300
gtgctmttgt gagaggcagt ccctgcgtgg aaagttgctg ggcccccagc ccccggctcc 360
cccgcacgca tgcatccact gcggccggag gaggccatgg agcccgagta gctgcctgga 420
tcgctcgacc tcgcatgcgc gccgcgtcgc ctctgggggg atgctgcacc gcgtttccat 480
agcagcatgt cctacggaaa cccagcacgt gtgtagagcc tcgatcgtca tctgctggtt 540
atttgttttt tcctttgttg ttttaaaggg gacaaaaaaa aaaaaaaaaa aa 592

<210> 55
<211> 504
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(504)
<223> n = A,T,C or G

<400> 55
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acttcaccct cctgcccgak cgctacgagc cactggagga gccggcgccg agcgagcagc 120
ccaggaagag gtaccggagg aagctgaaga agtactggca agaatgtcgg gaaggtcatc 180
atmaaakgat gccgctacgt ggtcatcggc ctgcaaggmt tcgctgcags ctactccgcc 240
ccgtttgcgg tagccaccag cgtggtatct ttcgtgcgct aatgggagct gctgtggcag 300
gtgcccccag agtgaacggg agcccctgct gtgggaactt tgtgaatcct ggagcatctc 360
atacttgaac acacakcata tttggaagag aaaacatgcc tttctttgnt gaatcacatt 420
agtatgatga gtgagtcatn cctgcccatc tgctgagctt ctcacatctc tcagtcacac 480
gtggacccag tggtcaatcc tgca 504

<210> 56
<211> 749
<212> DNA
<213> Homo sapien

<400> 56
gtaagataca agtttcaggc agggtctgaa ggactggatt ggccaaacat cagacctgtc 60
ttccaaggag accaagtcct ggctacatcc cagcctgtgg ttacagtgca gacaggccat 120
gtgagccacc gctgccagca cagagcgtcc ttccccctgt agactagtgc cgtagggagt 180
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gttgggagcc ctttggagaa cgccagtctc ccaggccccc tgcatctatc gagtttgcaa 420
tgtcacaacc tctctgatct tgtgctcagc atgattcttt aatagaagtt ttattttttc 480
gtgcactctg ctaatcatgt gggtgagcca gtggaacagc gggagacctg tgctagtttt 540
acagattgcc tcctaatgac gcggctcaaa aggaaaccaa gtgttcagga gttgtttctg 600
acccactgat ctctactacc acaaggaaaa tagtttagga gaaaccagct tttactgttt 660
ttgaaaaatt acagcttcac cctgtcaagt taacaaggaa tgcctgtgcc aataaaaggt 720
ttctccaaaa aaaaaaaaaa aaaaaaaaa 749

<210> 57
<211> 673
<212> DNA

<213> Homo sapien

<400> 57
agatgtcatg acccatcaac gtctcctctc attgtccctc tgctgggaca aagtgcttgg 60
tgtctggctg ggggacaacc aagagccccc aagtgcactt ccctaaggtc ctccagtgct 120
tgaacatcag cgtgctaagt cagaaaaggt gcgaggatgc ttacccgaga cagatagatg 180
acaccatgtt ctgcgccggt gacaaagcag gtagagactc ctgccagggt gattctgggg 240
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cccggcccaa cagaccgggt gtctacacga acctctgcaa gttcaccaag tggatccagg 360
aaaccatcca ggccaactcc tgagtcatcc caggactcag cacaccggca tccccacctg 420
ctgcagggac agccctgaca ctcctttcag accctcattc cttcccagag atgttgagaa 480
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ttgggctgac cgtgtctctc tagttgaacc ctgggaacaa tttccaaaac tgtccagggc 600
gggggttgcg tctcaatctc cctggggcac tttcatcctc aagctcaggg cccatccctt 660
ctctgcagct ctg 673

<210> 58
<211> 994
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(994)
<223> n = A,T,C or G

<400> 58
gacgggcccg gtgctgaagg gcagggaaca acttgatggt gctactttga actgcttttc 60
ttttctcctt tttgcacaaa gagtctcatg tctgatattt agacatgatg agctttgtgc 120
aaaaggggag ctggctactt ctcgctctgc ttcatcccac tattattttg gcacaacagg 180
aagctgttga aggaggatgt tcccatcttg gtcagtccta tgcggataga gatgtctgga 240
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tatgtgacga tcaagaatta gactgcccca acccagaaat tccatttgga gaatgttgtg 360
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ctggtcctca gaactattct ccccagtatg attcatatga tgtcaagtct ggagtagcag 600
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gtacatctgg tcatcctggt tcccctggat ctccaggata ccaaggaccc cctggtgaac 720
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gacctccagg tatcaaaggt ccagctggga tacctggatt ccctggtatg aaaggacaca 900
gaggcttcga tggacgaaat ggagaaaagg gtgaaacagg tgctcctgga ttaaagggtg 960
aaaaggtctt ccaggcgaaa aggagcnaca agtt 994

<210> 59
<211> 639
<212> DNA
<213> Homo sapien

<400> 59
tggtggtggt ggaggacctg cactctggcg cccagcagca ctggtccggc cactctgcgg 60
agatctccac gctggccctc agccacagtg cccaggtcct ggcctctgcc tcgggccgaa 120
gcagcacgac cgcccattgt cagatccgcg tctgggacgt gtctggcggc ctctgccagc 180
atctcatttt cccccatagc accaccgtgc tggccctggc cttctcacca gatgacaggc 240

ttcttgtcac actgggggac cacgatggcc gcaccctcgc cctgtggggc acggccacct 300
atgacctcgt gtcctccacc cgcctcccgg agccggtgca tggtgtggcc ttcaacccct 360
gggacgccgg cgagctcacc tgtgtgggcc agggcactgt caccttctgg ctccttcagc 420
agcgtggggc agacatcagc cttcaggtgc gtcgagagcc agtcccagag gcagtggggg 480
ctggagagct gacctcgctc tgctacgggg cacctcccct gctctattgt ggcaccagct 540
ctggccaggt ctgtgtctgg gacacgcgtg ccggccgctg cttcttgtcc tgggaggcgg 600
atgacggtgg cattgggctg ttgctgttct cgggttctc 639

<210> 60
<211> 470
<212> DNA
<213> Homo sapien

<400> 60
ggcacgaggt ttttattctt tgcacaattg tttcattgtt tgacacttaa tgcactcgtc 60
atttgcatac gacagtagca ttctgaccac acttgtacgc tgtaacctca tctacttctg 120
atgtttttaa aaaatgactt ttaacaagga gagggaaaag aaacccacta aattttgctt 180
tgtttccttg aagaatgtgg caacactgtt ttgtgatttt atttgtgcag gtcatgcaca 240
cagttttgat aaagggcagt aacaagtatt ggggcctatt tttttttttt tccacaaggc 300
attctctaaa gctatgtgaa attttctctg cacctctgta cagagaatac acctgcccct 360
gtatatcctt ttttcccctc ccctccctcc cagtggtact tctactaaat tgttgtcttg 420
ttttttattt tttaaataaa ctgacaaatg acaaaaaaaa aaaaaaaaaa 470

<210> 61
<211> 535
<212> DNA
<213> Homo sapien

<400> 61
ggcacgaggg gttcttgggc caatttcttg ctgactcagc cccactctgt gtcggagtct 60
ccggggaaga cggtaaccat ctcctgcacc ggcaacagag gcagcattgc cgccaactat 120
gtgcagtggt accagcagcg cccgggcagt gcccccacca ctgtgatcta tgaagataac 180
caaagaccct ctggggtccc tgatcggttc tctggctcca tcgacagctc ctccaactca 240
gcctccctca ctatctctgg actgaagcct gaggacgagg ctgactacta ctgtcagtct 300
tatgagagca gcaatcattg ggtgttcggc ggagggacca agctgaccgt cctaggtcag 360
cccaaggctg ccccctcggt cactctgttc ccaccctcct ctgaggagct tcaagccaac 420
aaggccacac tggtgtgtct cataagtgac ttctacccgg gagccgtgac agtggcctgg 480
aaggcagata gcagccccgt caaggcggga gtggagacca ccacaccctc caaac 535

<210> 62
<211> 696
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(696)
<223> n = A,T,C or G

<400> 62
gccagtatcc aatggggaat cagctctaaa aggaaccaga ccaacgtttt ccagcccntt 60
cattntggtg actgagggga ggaaagaatg ggagggggta ttcttgtcta gtggatggaa 120
aggaaacaca ctgtcaaatt actatatctc cttggttttc tattacagta gaattctcca 180
gccatatttt tattgtctat gggggaagtt ggagatggtg accttgatta gaagtgtctg 240
gagggggata aatggagggg ataagattca gttggttttg gaaaatgtta aagtcttaaa 300

```
ataatgcgtc catctgaaga atttttctta aaaccagagt ttataaaaat atcactgata     360
cagcctgccc cctcatttcc ctgccacagg agatgtcttg gactagagac acttgtttaa     420
taatagcttg tctctgatat tcccagtagc ttccctctgt gtgaggaaag gatagaaatg     480
ttcaggacat catcatacag gctcctcatc tacaaagttc cagtagcagt gacgcctaca     540
cggaagactt ggaactgcaa acaggctggg gtcacctcag tgacatctga cgctgtccaa     600
ccagaagttc gatttttgtt ctgggggtga aggaggaaac agacgtgtac taaaggacta     660
aaataatttg tctatacaaa aaaaaaaaaa aaaaaa                               696
```

<210> 63
<211> 256
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(256)
<223> n = A,T,C or G

<400> 63

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taatatttat tgaagnccta ctttgtcctc tacatagggt agcttctgtc agggaatgtt      60
ggttcttccc aagaaacact gattttcttt cagggagact tcatgtgttc atttatttcc     120
accacagcag atttaagaa attataatat gtaatatttg atatctataa agagtanatc      180
taacgtgaat aaattatgaa gcatactaat gagtacctat gacccatnac acatatacat     240
taaaacattt taaata                                                     256
```

<210> 64
<211> 678
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(678)
<223> n = A,T,C or G

<400> 64

```
ggcacgaggg ctanaagcca tcgcatcttt ctccgtattc aacaagccca ttataacaag      60
tctttcacgc tcaaccattc aaacaatccc aacaatccca gcctaagaat ttcctggcat     120
ttgatgaaaa tggctgtggg ttttgctatc tttaagctcc ctgggaagca ggatataagc     180
ccagggctgg caggctcttt ctagctacct gctcttgcac atagagtttg cctcatggtt     240
gcaagaaggc tgccatagct ccagatggca catagacatt ccaggcagca ggaaaaagga     300
aggagcactt aaccgaagga ggtcagggag ttggttagtc tccacctgaa gaagagagcc     360
atgaaccagc ttcagttgac taacgggctc ctgtgagtgc atctttggac ttttctggag     420
gttgaaatct agatgtggta tgtgtcttaa agcagccacc aactcctccc attaccttcc     480
aagtgagccc aactacacga tggagcctct tccctgccct tggatctggg ctgagcctct     540
gacttgcgtt gaccaacaga atgcagtgaa agtgatgccg atactaccct ccctgcccta     600
gacttgggat acctgcagct atattcttcc attcctcagg acttgcaaaa acgggtctca     660
ncatgccttn ccagagcc                                                   678
```

<210> 65
<211> 678
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(678)
<223> n = A,T,C or G

<400> 65
ggcacgagga ggacacactc aggatagaaa ctttatgtgt atagggaatg tggtacagcc 60
tttagccagg agtcatactt catcagacac cagaggacac acacagtgct gtggcttttt 120
cagccattgc tagataccaa agtggagaca ttctgtgtgt gattatgcat gagactgtac 180
tggtaagact tgtatctcca tccacctgaa ggagaattgc tggctcattt tcaggagccc 240
tgcccttcct cactgtggat ggtgggttgt ggaaacccgg tcaggtaatg atagtggcag 300
gaggcagtca aatgcccagg cagatagggg tgggtacctg gtgaaaccca accttaaagc 360
tgaagacagt cccggctaaa tcctcatact gaattgagaa cctgtcttcc catttggtgt 420
gctttcctcc gattgatccc aacccttcac ctattttacg tatacctgcc ctttcctaat 480
tggtttttac actgctgtgc ccacctttg agtggtgcct ttgcatactt acaaatcagt 540
caacgtgtat tcccctattc tgagcccata aaagacccan actcagctgc agtgaggaga 600
gaaatcccct gctgnggggg gtggggacca ctccctgcat ccctctncac tganagctgt 660
ctttttgctc aataaaat 678

<210> 66
<211> 606
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(606)
<223> n = A,T,C or G

<400> 66
gcatcaactg ccgccagccc ttcatnttnt ccgcctcttc ctagacgtgc tacacctggt 60
tgagttctac ctggaggaag ggatcactga tgaagaagcc atctccctca tcgacctgga 120
ggtgctgaga cccaagcggg atgacagaca gctagagatt gcaaacaaca gctcccagat 180
tctgcggcta gtggagacca aggactccat cggagatgag gacccgttca cagctaagct 240
gagctttgag caaggtggct cagagttcgt gccagtggtg gtgagccggc tggtgctgcg 300
ctccatgagc cgccgggatg tcctcatcaa gcgatggccc ccacccctga ggtggcaata 360
cttccgctca ctgctgcctg acgcctccat taccatgtgc ccctcctgct tccagatgtt 420
ccattntgag gactatgagt tgctggtgct tcagcatggc tgctgcccct actgccgcag 480
gtgcaaggat gaccctggcc catgaccagc atcctgggga cggcctgcac cctctgcccg 540
ccttggggtc tgctgggctg tgaaggagaa taaagagtta aactgtcaaa aaaaaaaaaa 600
aaaaaa 606

<210> 67
<211> 579
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(579)
<223> n = A,T,C or G

<400> 67
ggcacnaggc ccaattatat gttcattttg tatatttttt ggtcggggga aaaattgacc 60
tgcagtanaa aaacctttga ccatttttat gtccattgga tactttcctt tttatcatct 120
taaaaaaaga taactagtac taatcattgt agtggcctaa gtgtgattta actcttgaag 180

tcacaccctc cgaaagatga gtagaaacca gcaccagcac agcccagatc ttctctttcc 240
tctccttttc ctcatttatt cctaaaggaa tctgaccatt ttacgtctct acggcccaaa 300
aaaagacaaa aataaaaatt cctttttatt cctgtcaact ggatggaaac acaaatttca 360
tggagctgtg taccatcgaa gaaacctggt gtctggcatg aaattactgt aaagaacttc 420
ctgtaaaaca cgttctttaa caaactgaaa tgaaaagcat tggagcgtct gaatgaaaga 480
cgtgacctcc tgctgggact ctgatggtct tcagcattca ccttcgtgtg tcttcagtgt 540
ctcattgtca tccctgcttc tgtttggtct tagagtgtt 579

<210> 68
<211> 258
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(258)
<223> n = A,T,C or G

<400> 68
ggcatntggc cctgaccana tccttaaccc tagcgatggc ttgggagatg gggggttgga 60
tagcattntc tttcttggcc cttccttatc ctaggaaaan atggttcctc tccttgtgtg 120
tgtctcttcc ccccacccct aattcttctg ctctgtttgg gaagacgtgg aggaaaaggt 180
gacttctgcc cccaccgctc ttaccccccac tgtantggcc tttggagatg cccccacctc 240
ccccccacca actctcgc 258

<210> 69
<211> 628
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(628)
<223> n = A,T,C or G

<400> 69
ggcacnagga gaattntttg gaataaaact gactgataaa actggaaatg ttgctcgggc 60
tgttaactgg caggaaagat ttcagcatct gaatgagtcc cngcacaact atttaagaat 120
cactcgtatt cttaaaagcc ttggtgagct tggatatgaa agttttaaat ctcctcttgt 180
aaaatttatt cttcatgaag ctcttgtgga gaatactatt cccaatatta agcagagtgc 240
tctagagtat tttgtttata caattagaga cagaagagaa aggagaaagc tcctgcggtt 300
cgcccagaaa cactacacgc cttcagagaa ctttatctgg ggaccgcctc gaaaagaaca 360
gtcggaggga agcaaagccc agaaaatgtc ttcccctctc gcctccagtc ataacagtca 420
aacttctatg cacaaaaaag ccaaggactc caaaaattcc tcctcagctg ttcatttaaa 480
tagcaaaaca gctgaagaca aaaaagtggc accaaaagag cctgtggaag agacagacag 540
gcccacccan agcccagcaa tgaagctgcc aagccaagaa atacagaaga aggacagtaa 600
tgctganaac atgaattctc aacctgag 628

<210> 70
<211> 439
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature

<222> (1)...(439)
<223> n = A,T,C or G

<400> 70
ggcacgaggc gcacacccgc tgggcggccc ctgtggagac cctggaaaac atcatcgcca 60
ctgtagacac gaggctgcct gagttctcag agctgcaggg ctgtttccgg gaggagctca 120
tggaggcctt gcacctgcac ctggtgaagg agtacatcat ccaactcagc aaggggcgcc 180
tggtcctcaa gacggccgag cagcagcagc agctggctgg gtacatcctg gccaatgctg 240
acaccatcca gcacttctgc acccagcacg gctccccggc gacctggctg cagcctgctc 300
tccctacgct ggccgagatc attcgcctgc aggaccccag tgccatcaag attgaggtgg 360
ccacttatgc cacctgctac cctgacttca gcaaaggcca cctgancgct atcctggcca 420
tcaaggggaa cctatccaa 439

<210> 71
<211> 328
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(328)
<223> n = A,T,C or G

<400> 71
tnggaactca ggtttacnca aactgagggg gccccagccc tngtaccncc cctgttaccc 60
caggatccat ntgccctcan aaaagngttc aggtacagca gctgaggctg ccctgaggaa 120
tcaaggggcc attaccaagg ggcaggaaaa ggatatgtaa naggnggcct tcatggtana 180
gcttgaccca anaactactc cncattngga tggcccagac tgactccatc ccctgacttt 240
ccctttgact tcnccctgtt tgtaaataaa acaataaaat ggaaggtgct gtggacggga 300
aaaaaaaaaa aaaaaaaaaa aaaaaaa 328

<210> 72
<211> 721
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(721)
<223> n = A,T,C or G

<400> 72
ggtttctggt gctattctgc caagttatcg agctcctcct catgtttcaa cattccatct 60
tcccgtttct atcctcgact ccaaagtaag ccttcttagc tccaatcagg gatgaggggc 120
tcaacctctt ctgtcctcaa agaggccaaa cgcagtgcca cagtcggtag ccttcacttt 180
tagatgtcct attcatgtaa aaaagaaggt gcccccacca ggcttacatc agcaataagc 240
aattctaatg caacgatggt gtccacattt taccccagtg tgtgcccatg tatgcctttg 300
tgcccgtgta attattgtta gcgccccttt cacttagagg ggtgatgata aactgtggcc 360
accttgatta caacccacat ttcctgcttt ggggagcttc caagtaacag gccatttctt 420
acctccctcc aggaacagtg ggcactgccc accacctcgt gtctgctcat aggatgacgc 480
tggagatccc cacacttact ctaccctctt ggcaaattgg cattccggtg gtggtttttg 540
tttcctttaa cacattaaat aaatgagtat ataggatgtg aggggagggg tgagaacaac 600
tagctgtagc atgtgtaggc tatatacttt accattcgac ttctttnctt tttttttttt 660
aaaaaaaaaa aagtgcttga ctgggttcaa gcttcatcat gaaaaaaaaa aaaaaaaaaa 720
a 721

<210> 73
<211> 596
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(596)
<223> n = A,T,C or G

<400> 73
attgtacaga acagcccagg tagggaggac agntgcccca ggtcccatag gantgcatgc 60
ctcaagccca cgtcatgcag agccactcag ctcaccctgn tcagggcacg tggtttacct 120
gcattcccct nttgcaggtc ctacgtgtgg aggacgtacc atntgacctn tgcaggagag 180
aaactcacgg aagacagaaa gaagctccga gactacggca tccggaatcg anacgaggtt 240
tccttcatca aaaagctgag gcaaaagtga gcctccagac aggacaaccc tnttcatcac 300
tggtggctga gctttttccc agcaggaatg ggtcctcgaa tcatcgtgcc tntttcacan 360
aaaggacgtt gtggtggcct caccccaggc atgcccaaca gtaactgtca gcataaacct 420
gggggccctc aggactagga cagggtgagc cagtgctccc tcctttcatg tacttggcct 480
gagactgacc tctccctagg tccaaatgcc ctagtcacat ggcagaccca cggcctggcc 540
cactgtataa aataaacctg tttgcttntt agtttgaaaa aaaaaaaaaa aaaaaa 596

<210> 74
<211> 302
<212> DNA
<213> Homo sapien

<400> 74
ggcacgaggc ttaaaccaag tttctctgca gctctttcgg ttctgcttac agtgtgtggg 60
aaatctgatt tttttcccct agtaatagtt tgataagaaa tttagtgtat tgactgcctc 120
agtgacacaa tttatcttta aaggtgtgga agctggtggg gaccaaatgt tacctgtgtt 180
tttgctgttg attgctattt tcagaagcaa accatgtttt tcacttacag taggagtcaa 240
caaatttggg attttagaag ggggaggagg gagctatttg tgtaagactg ctgtcatatt 300
tg 302

<210> 75
<211> 635
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(635)
<223> n = A,T,C or G

<400> 75
ggcacgaggt ttaatgtgac tttcctgagt taacactgaa tagtatttct aaaatttttt 60
gtactttatt ttttaatgta acttgttcta tctatctata tatatatttg atagtttgtg 120
gaataatatc ccccagtatt ttccatatta aatgctaatt atctttttgat ttcttttttca 180
taagcagatc tggcatttat tacagggctg ccgcttaaga gaactcatta taatgaacgt 240
ttattatatt ttgcagttcc atgcctgttg tccattgatt gacatgagca cccctgtttt 300
ctctggagaa atacctcccc tctctggggt gcttcctgtg gtagtgtctt tcaggtatcc 360
gttccactag ctacaggtga gcattttacc cattgttgga taatggtaat ctctttttca 420
gaattttgag tctgtaattc atttgtacat gaaccagaaa atgtgggaac tcattcattc 480

ttgtcccaga attctgttga gaacatccat tcattctggc taattgatta caagaataac 540
tgnggatacg atccctttan aacctgcttc tctgatctgn gtgtttcctc acttctcaat 600
aaaaatgtct tttgctaaaa aaaaaaaaaa aaaaa 635

<210> 76
<211> 678
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(678)
<223> n = A,T,C or G

<400> 76
ggcacgaggc tcttgattct tggcttgcct ctcctccaat tccaaactta gtgaaatggc 60
cttaagcatt ttaaactgta tgtatacatt agcgcattca tgcctttcta aacgcatttc 120
aaatgtcaac caggaaggca caccactgta ttagttttat actgccgctg taaaatttac 180
cacaaactta gtgacttaac acaaatttat tgcaattctg taggctggaa gtctgactat 240
gggtctcact ggactagaat caaggctggc aggctgcctt ccttcctgga ggttctaggg 300
gagactctgt ctcctgctcc ttcaggctgc tggcagaatc cacatccttt cggtggcagg 360
gccaaggtcc ccactttctt gctgactgta aactaaggcc acttccagct tgtagaggct 420
gcctacattc cttggctctt ggccccctcc tccatcttca gagctagcag gttcagtctg 480
tgtcacgaac catttctctg gttccctgca gacaggaaag gttgtcccta aggactcatg 540
agattaggtt gggcccagcc agataataca tgataatctc cctcctcaag gnttttaata 600
ttaaacacat ctgcaggaca cattttgcca tgtaactaac attcactggt ccaggggatt 660
aaggaatgaa ccctcttt 678

<210> 77
<211> 669
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(669)
<223> n = A,T,C or G

<400> 77
ggcacgaggg agaatcttaa aaaaaaaaaa acgtttctca ctgtcttaaa tagaattttt 60
aaatagtata tattcagtgg cattttggag aacaaagtga atttacttcg acttcttaaa 120
tttttgtaaa agactataag tttagacatc tttctcattc aaatttaaag atatctttct 180
cctcttgatc aatctatcaa tattgataga agtcacacta gtatatacca tttaatacat 240
ttacactttc ttatttaaga agatattgaa tgcaaaataa ttgacatata gaactttaca 300
aacatatgtc caaggactct aaattgagac tcttccacat gtacaatctc atcatcctga 360
agcctataat gaagaaaaag atctagaaac tgagttgtgg agctgactct aatcaaatgt 420
gatgattgga attagaccat ttggcctttg aactttcata ggaaaaatga cccaacattt 480
cttagcatga gctacctcat ctctagaagc tgggatggac ttactattct tggttatatt 540
ttanatactg aanggngcta tgcttctgtt attattccaa gactggagat aggcagggct 600
aaaaagggat tattattttc ctttaatgat ggggctaaaa ttcttcctat aaaattcctt 660
aaaataagg 669

<210> 78
<211> 134
<212> DNA

<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(134)
<223> n = A,T,C or G

<400> 78
ggcacgaggg gtcgatttta atagcgaatc ctttttcttg tagaggtaag taaaatcttc 60
ctgacaaggt tgtcctctnt tcacggcaca gacaatgggc ggnctgttta tgaggggtga 120
gaagngacnc ccgc 134

<210> 79
<211> 396
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(396)
<223> n = A,T,C or G

<400> 79
tanatgcaag tntnttgtng gatatacgta ttgagatatt acncctagtc tgtggcttga 60
ctgtttcctt tatgtctttt gatgaatana agttttaaat tttgacaagg tcaaatttat 120
ttttttcttt tgtttgatat tttttntctc caatttaacc ccaagatttc anatattntg 180
ctctattana taaactttat atttttatat ttgtgatctn ccttgaattg atatgtatgt 240
tgtgaattat ggatcagggt tntttttttc ccccatacaa gtatccagtc attgtaacnc 300
tgtttattga aanaattatc ctttcctcat taaattncct tgccaattag taaaaaatca 360
attacccata aaaaaaaaaa aaaaaaaaaa aaaaaa 396

<210> 80
<211> 731
<212> DNA
<213> Homo sapien

<400> 80
tctacatcat cctgagagcg ccttcagact ggacagaact gtaggaactg ccgtctctta 60
tggtagatgt ggcccatcac aatatacctg caggtttaaa ctctggacac ggcttattgc 120
agctggagga gtccagggct aacatgtgga ctcggaagaa gaagccgtcg ggggtgaggt 180
acaggcggtt catcttgtac agcccgtccc gatccaccag cagggtcacc agccggatgc 240
ccgcgcccag cacgtccaca tcgtgcacga tcccgttcac cgcgaattcg ctctcgcaga 300
acgcctcgcg ttcgtccaga tcggaccggc aggcgcgcgc gcagggctca gcgccgggct 360
ccgcgtccct ctgcggcggg ctgaggccga agctgaggct gaagcgcggc ggcggcgcgg 420
gcgcgggtcc cggggccagc gcggcggcgc gcggccggtt ggggtgggcg tggccggcgg 480
agccctcggt ggcgagcgcg ggcggccggg cggcggggaa gcgcagggcc cgtgagcgcg 540
cgcgtcgcgg ggcgtcctcc gacgcgggct cgcggcgcgg cgacgcgcgg ttctcggctt 600
gcgccaggcg cgggcccggc gggccagcgg gcgcgcgtgc ggcctcccgg aagctggcct 660
ggttgtcggc cggcggtggg ggcttccggc ggcgcgggtc aggccaggcg cggacggcgg 720
gagcgacctc g 731

<210> 81
<211> 396
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(396)
<223> n = A,T,C or G

<400> 81
cccttcatcc caatccntga gaagttcttt ttctgcttcc tgcccaacat natcaacagg 60
acctatttcc cattttcctg ntcttncntn anccagntat tggnngnggt tttgaaatgg 120
ctnatnatga ggaanagtnt gatccnacac ttggagganc naggggtgca ggtggtnttt 180
tggtgcctta atgaanagtc ggattttgaa gcagccttna gcgtgggagc cacnggcgtc 240
ataacggatt atcccnnagc cctnnggcan tacctggaca accatggncc agctgcccgg 300
acctcctaag tccagaancc tngaggtntc ctgtttntnt tccatgaaaa ataaatattt 360
gcctttcgat caaaaaaaaa aaaaaaaaaa aaaaaa 396

<210> 82
<211> 502
<212> DNA
<213> Homo sapien

<400> 82
ctcagaggaa agaagaaaag ggccaggagt cagacgtcac acccgggggc tccccttcc 60
catgtagaag tgttggcatg cgtcagtctc ctttacagag gggtggatgt atccgtggag 120
gaggggcctt ctctctttct aattgcacta tacttgttct agcttcagtc tggagatact 180
taagacctcc atggggtcgt gatccataga cttagcaagt cttgccttat ctatggagct 240
cgatggtgag aattgtgacc attgtctgat gtccatagtt ccttccccct agattgtttc 300
tttccacgga ttgtgttcat ctgaaccatt ttatttttta tttaccaaag tactgtactt 360
ggctatttgc agtgttttca aaaccaaatg tttctttttt tgtgttttta atcttcgata 420
cttggtgcaa tagaagctgc aaagatgtgc cactttatct atgaaatgga gttttgtata 480
ccaataaatt ctagtttaaa aa 502

<210> 83
<211> 666
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(666)
<223> n = A,T,C or G

<400> 83
gatttttggt atttgtactt atttatgaac tttacttgag acagaatatg gttaaaatta 60
ggaacatcta ctttgaatga ggtttatttt tctattttga atttgcctta tgtatattca 120
aaggcttatg gaaatactgt aaaggaacat taggaaaagg acaaataggc tataaccatc 180
tatcttaaaa tcagaccctt agtataagca cctctttttc ttttcctctt tgacaattta 240
gtctcttatt taggtccatg taattaattt cattccatta ttttttagct gtttattcta 300
aaaaacaaaa attttcagcc actcccattt attctcccat gacatggtcc tatatagcag 360
tacttaacac agtgccttgc acatagtagg cattcagtaa atacttacat gcatgaatga 420
ataatgtatt ttcagtgtaa caaatttatt ataaaagtgt agttcgactc ttcttggtct 480
tggagtttga gagtacagaa ttacagggaa tgaagagagg tataagtaga tattttaatg 540
gaaatgaagt ataaattaat aacttgactt acccctccat aagttactgc taactggaga 600
taccttgta tgcccaacct gtaaaggaaa aagntctatt tctatattta taggacattc 660
ttctca 666

<210> 84
<211> 199
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(199)
<223> n = A,T,C or G

<400> 84
aanntactta agttattcaa agaatgttat ctttcttgca agagtaattt aagcacatgg 60
gaaagattct agactttttg tttcttgcaa canacagtgc cctctgctgc tagaaacctt 120
tttctactta ctatcatttt tattgtggct tgagctcanc tcaatctggt gcagatgacg 180
ctggacaact actaaccaa 199

<210> 85
<211> 670
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(670)
<223> n = A,T,C or G

<400> 85
cacttcagga gagaacttca tagcacaatg tctttctata agatattttt aatgatttag 60
tattttacaa catttgttta ccatattttg atataccatt tttttctatc tgcccagttt 120
tattaaaaaa actatatatt attttctaaa gaaacaatca tatttttata caaaattatg 180
ttttcaggta acgaaataga tgtagggtac agtggaacat aagcagtgtt acccctggct 240
gggagtcagt attatacaac aaatggtgag ctggaacatg ccctgtctgt gctgtccctc 300
ctgtgctggg tcgcggatgt gtaggcaaca ttgccttatc acgctaggtt cacctgacac 360
tttaaaagga aaaaaagttc catagagttc tgtggtcaca aaattgtttt gcttttatca 420
aatactttaa tagaaccaaa gttgcagata ttggaatgta tggaagtatc tcagtctctg 480
cataagagga ttaaagtatg aaaggatcat ttaatgactg ttttacttat aagtcattaa 540
gtaatccacc atttcttatg gatgatgctt aagcctggtg aggtttgtac tctaaggagc 600
ccagatcata atgcagngca tttccttanc ccttagagtt tcttgcaaac atttaaaaaa 660
agacntattt 670

<210> 86
<211> 401
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(401)
<223> n = A,T,C or G

<400> 86
atcttcccac gagtgggatt ctggccttca gagaccagga gggagtgtct gggccgcang 60
tgtggcactg tggtgagagt gtgtgtcttt gcacacacag tgcagcggga acggtggggc 120
tggctggtgc tgaagacaga cacactcctg agccaaggtc ttgtcttcaa cctccccgtc 180
ccgttgtccc attttgctct gtgaaggtgc aaatcccttt cttcccttcc catctcaggc 240

tctcctgttt tccctcaggg tccagtatgc ctttgagctt tagctgttaa aaaggaaccc 300
ccgtgacttg acacagcttt cacanctggc tgctangacc ggcgggctgg gtgttcacgt 360
gtgtctgtgt catggatgca atgcangccc tgnangactg t 401

<210> 87
<211> 373
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(373)
<223> n = A,T,C or G

<400> 87
cttttttttt cttttttta tttgaaaact actttancaa taattaattc catgattatc 60
acattctgcc attaagggat attagtaccg taatactgaa gaaattttat taagtctgaa 120
cttctggggt aggcagcttc tttgtttctt ttctatccac ccttgtcggt tgaggtattt 180
gtttcttgac taataaaccc tttgatactt taaccagaaa tcagctcata aagctatttt 240
tgagtatagt tngggnaaaa aaaaaaggtt aacttgggna ataccttcca nnctgacctc 300
cntntaccaa gatatttttc agggctttta tttactatgc nctaanacta tgcncttttt 360
ctgaaatatt ttn 373

<210> 88
<211> 507
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(507)
<223> n = A,T,C or G

<400> 88
ccaggagcag cccattcctg atgcttcttc agagactcct gcaggcagcc aggccacang 60
acccttgtgg tcccacccca cacacgccag attctttcct gaggctgggc tcccttccca 120
cctctctcac tccttgaaaa cactgttctc tgccctccaa gaccttctcc ttcacctttg 180
tccccaccgc agacaggacc agggatttcc atgatgtttt ccatgagtcc cctgtttgtt 240
tctgaaaggg acgctacccg ggaaggggc tgggacatgg gaaaggggaa gttgtaggca 300
taaagtcagg ggttcccttt tttggctgct gaaggctcga gcatgcctgg atggggctgc 360
accggctggc ctggcccctc agggtccctg gtggcagctc acctctccct tggattgtcc 420
ccgaccccttg ccgtctacct gaggggcctc ttatgggctg ggttctaccc aggtgctagg 480
aacactcctt cacagatggg tgcttgg 507

<210> 89
<211> 796
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(796)
<223> n = A,T,C or G

<400> 89

gactttaatg ggnatatggg ctctacactc atagggaccc acatgttcca agcctccagc 60
tgtgtgttct gcttcctcaa gcagcagctg actctgatat tccccatctc accttagcag 120
tctatatcaa gcaaagggca tgcagaatcc catgcaccca tcatgtatgt aataaaaggc 180
agatattatg tggtctcttg aaccagtctt aggcatggag gttgaggatc aggagtgact 240
tgagggtact gactggcaga gcaggagccc cgttattttg gacaaacacc gccactttaa 300
gtttcagctt catttttagc cttctggatt taaggaaatt actttttttaa aaactataag 360
cagccaaaaa aagcagacag taaaatgcag ataaaacagc tcgggcacag aggaaggtgg 420
aggaaaagtc tcttgggtaa ctgccaaact tcaccctcat acaatgggcc ccagtaaaac 480
agtgggcctt aataagcaca ttcctttccc tccaggtgca ctaaaatagg gaagctaaaa 540
gcagacttgg ggggtatgcc tacagctgca gaaaaatgta taaaagcaaa cacaccactc 600
tccctcccat ataagcacaa caaaaaaaca cagaagcagt ccaagcctnt aagaaactct 660
cccaccctaa atccttaaac actcttagtc tgtagaaaag actctaacct aattcagcca 720
gcagcccctc tcaggtgtgt tttntntaaa ataaacctgt nttaaccatc aagccaaaaa 780
aaaaaaaaaa aaaaaa 796

<210> 90
<211> 462
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(462)
<223> n = A,T,C or G

<400> 90
tagaacatnc tgaatttttt ttgtactgnt ggactntatt cagtgtcatg tcctatatct 60
gatcaagttn tcaagnagat aattttanaa tgaaaaagaa aatcctcttg tnggaaacaa 120
aagacgtttt atatgtgcag tatgacaaan aggagtttca nagacaactt tgaatccttg 180
tcagcctgga gaccagcncc agaggaatnn ccaaggcaaa ctcccatata tttgcttccc 240
ccaaattgct gcccctacag actcaaagct ctttttcttt gttttgttgt ttntctaaaa 300
atttactgtt ntttgtcgat gctatataag ccagggagtt ttaagacgcc agctntttga 360
natttgntca ttcccctgta tttcccacat anatattaca tatacccgng taataaattt 420
atgtttgtta aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 462

<210> 91
<211> 591
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(591)
<223> n = A,T,C or G

<400> 91
cgggccttgg aagcctttga cctggatcct gcacagtggg gagtcaatgt ccagccctac 60
tccgggtccc cagccaacct ggccgtctac acagcccttc tgcaacctca cgaccggatc 120
atggggctgg acctgccccga tgggggccat ctcacccacg gctacatgtc tgacgtcaag 180
cggatatcag ccacgtccat cttcttcgag tctatgccct ataagctcaa ccccaaaact 240
ggcctcattg actacaacca gctggcactg actgctcgac ttttccggcc acggctcatc 300
atagctggca ccagcgccta tgctcgcctc attgactacg cccgcatgag agaggtgtgt 360
gatgaagtca aagcacacct gctggcagac atggcccaca tcagtggcct ggtggctgcc 420
aaggtgattc cctcgccttt caagcacgca gacatcgtca ccaccactac tcacaagact 480
cttcgagggg ccaggtcagg gctcatcttc taccggaaag gggngaaggc tgtggacccc 540

aagactggcc gggagatccc ttacacattt gaggaccgaa tcaactttgc c 591

<210> 92
<211> 647
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(647)
<223> n = A,T,C or G

<400> 92
gaataactag aaatttattg gatcaggttt cacatttgca tttttgaaaa ctactaccaa 60
aaagatttca ccaatttaca actccatcat tagtaagaat gcctgtttgc ctatagtctg 120
ccaaccctga atccttaaaa atttttgcca atctggtagg caaaatttct ttcttttctt 180
tgaatattaa tgaggaggaa catcttttca tgtttcttgg ccatttgcat ttcctattat 240
gaattgcttt tgcccatttt ccttttttta attatgaaag tctaatgact accttctcat 300
tgtataaaaa acacagttct ttgaatagag agacccttt ctccaatgct accaatcaca 360
ttccacttac cacagtttaa catacatcct ctagtcacct ttccgtacga atatacatac 420
acataaaaac actttttaca taaataggat ctcatattct gtagcttttt aaaatttttgg 480
nctcaaaaaa aganaacang gctttaaatt tctttaatgg gtgaatatga ataaatacta 540
tgaaaatgcc attatttatt cccttaattt ttttcctctc gctattacat tgccaaagna 600
aacatcctat tcanatgtct ttgngcatgt gngngaatat ttcttta 647

<210> 93
<211> 740
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(740)
<223> n = A,T,C or G

<400> 93
gtcatgatag aggaaattaa tgcaaggaaa gaaaacaagc ccagttggtt tggcttgtgc 60
tactgcaagg ctttacaatc agattatatc acatacatag atgaactcct gacctatatc 120
aatgcaaaac ccaacctgtt ctctatgctc ctaacggatc cacatctggc tctgaccgtc 180
ttctttggcc catgctcacc ataccagttc cgcttgactg gcccaggaaa atgggaagga 240
gccagaaatg ccatcatgac ccagtgggac cgaacattca aggtcatcaa agctcgagtt 300
gtacaagagt ctccatctcc ctttgaaagt ttcttaaag tctttagctt tctggctttg 360
cttgtggcta tttttctgat tttcctataa gtaaaagatc tcctaaatgg aagatgcaca 420
gagtagattt acaatgctcc aattcctctc ttacagcaat attgccttca cagttataaa 480
ctgtattcaa atagtaaagg ccaccctctc gcttccctgg ctggccccag ggctaccact 540
ggtattcctg agcctctccc agctccactt ctaatgctag agaatgataa ctaagacttc 600
tgtgcatttg aaggttgttg gaaagttaca ggttcatttt anaaagaaag ctgttcttga 660
cagcactctt gagccatcat acctctttcc ntataaacta ttttccagaa ctcnactaaa 720
accccttact tcacaaatga 740

<210> 94
<211> 608
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(608)
<223> n = A,T,C or G

<400> 94
ttttacaatc ctaggaaggc ccaccaattt catttcacgc gccagggcgg ctgcagttgg 60
aggccgaggg cagccctctg ctcactgaat gtcttgcatg tgctgactgc tgcccgcagt 120
gctgaacatg ccccaccgcc caggcccagc actgcttgtt gggtcagcat ctagtgctgc 180
tgtcacatct ttgtctgcac agccagtagg attgcctcag ccagggggtt tatcagaagg 240
tgtgcaaggc ctttgggga actgagcccc tatagtgggc agtctccttt accttcccac 300
ctccctgaaa agcacagaag acagtgcctt ggtttgtgtt ttgaagcaaa caagtcagct 360
ttctggcttt gccccaaaac tgtgatggaa cataataaaa ctggagatat ggtttttaac 420
actgcaaaaa ggaaaaagca tcaagtttct acttctggct ggaaagcaaa accaatctca 480
gctgacaagg ctgggcaaac taagttttcc tgagcccatt ttcctttgag ccctgaccta 540
ncctgcctta cctcattaag gtttggttaa agcantggaa aggagganga ngcangggtg 600
gatgggg 608

<210> 95
<211> 706
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(706)
<223> n = A,T,C or G

<400> 95
agaataatga gtacacaagt ttttgcctat ccctttcctt aaaatataag aaataaagga 60
gctcttacga aaaacccagg ttagaccgca taaaaaataa agtgaacagt gaggtggtag 120
caagacttct ttttagaaaa gaaagcattt acctgcctgt ctgtaaggtg gaaatttcat 180
cagtttgcaa acgataagaa atgcagactt gctcttgata gaaatgctta gaaacactct 240
gggggggaa aaagctcctt ccatatactg tgagacattt gttaagtgac atctattgtt 300
tatcagcttt taaggataaa aaaggtattt taaaagttgg atatttagga tatttgagga 360
tattccttta tgagctctcc atatccttct tgagaaactg gttaaaaaag gaataggggt 420
tgagtgttac agagagtagt ctgaagattc ctgtgtaaaa gcaaagctaa caagcaatga 480
agacatgaag caaaatacta atctaattgt gtaaagaaag gatattttaa taagttcttt 540
ctgcttgctg ctaagagttt gctaaagtgt catgaattat tctggttatt actaaagttt 600
ctatgaaaca agtagatttt aagaataaat gtttctggaa aagaactatg ttatgatttt 660
gtanaaatgt aaagattact tgaggtgttt aaaataaatt tttcat 706

<210> 96
<211> 719
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(719)
<223> n = A,T,C or G

<400> 96
ccagggcctg tccctgcccc agtggcggct gcgctgggtt caggcccagg gggccctgna 60
gaagctgtgc agctgaagag agggttcaaa cggaagccga gaacttgaca ctgttcaccc 120

```
caacacctca cctcccccag gacatttgga agaaagcagc gccaggattc ctcggcagtc      180
gtccccaccc gcacctgcag tcccctcatg tgctgttctg ctgccccact cagctcctgg      240
accctgtcct ttcatcccgc taaagcaccc cctaaaaccc cttcatcact ttcattctca      300
gcaaaaagta attgagcacc tcctctaggc gctggggagt ccacactgaa caaaagaaac      360
agaaaaccct gtcttccagc agttgagttc tagggcaggg agacagagtt tacaagataa      420
ggaaaatata tatgtagtat gctgcaagtt aactgctgtg tgggaaatcc agcagggggt      480
gggatgtgtg atttgaattg agggccacac tgcccaggtc gtgctccgtc aaggggtgag      540
caggagcaac aggggtggct gagtaanggc ttgcagctgg aggcaacagc acatgcaaag      600
gccctgagcc aggatgtgct gcaataangg ccactgaggg ggacagtgta ngttgggggg      660
tgangaatgt attaaaaaga ngaaaaattg ccttttaaaa aaaaaaaaaa aaaaaaaa        719
```

<210> 97
<211> 572
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(572)
<223> n = A,T,C or G

<400> 97

```
gcttggctaa tttccaaatt attgcataat atgttctacc ttaagaaaac aggtttatgt       60
aacaaagtaa tggtgttgaa tggatgatgt cagttcatgg gcctttagca tagttttaag      120
catccttttt tttttttttt ttttgaaagn gtgttagcat cttgttactc aaaggataag      180
acagacaata atacttcact gaatattaat aatctttact agtttacctc ctctgctctt      240
tgccaccccga taactggata tcttttcctt caaaggaccc taaactgatt gaaatttaag     300
atatgtatca aaaacattat ttcatttaat gcacatctgt tttgctgttt ttgagcagtg      360
tgcagtttag ggttcatgat aaatcattga accacatgtg taacaactga atgccaaatc      420
ttaaactcat tanaaaaata acaaattagg ttttgacacc cattcttaat tggaataatg      480
gatcaaaaat agnggntcat gaccttacca aacacccttg ctactaataa aatcaaataa      540
cccttagaag ggtatgtatt tttagttagg gg                                    572
```

<210> 98
<211> 520
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(520)
<223> n = A,T,C or G

<400> 98

```
tcagcctggg gtcctccact tctggaggcc tctaggctct atcagcaact gggggacana       60
acagcagagc tggagagtct ggagctgcta gttgaggcct tgaatgtccc atgcagttcc      120
aaagccccgc agtttctcat tgaggtagaa ttactactgc caccacctga cctagcctca      180
ccccttcatt gtggcactca gagccagacc aagcacatac tagcaagcag ggcaggagac      240
gctgcagagc attacttgga cctgctggcc ctgttgctgg atagctcgga gccaaggttc      300
tccccacccc cctcccctcc agggccctgt atgcctgagg tgtttttgga ggcagcggta      360
gcactgatcc aggcaggcag agcccaagat gccttgactc tatgtgagga gttgctcanc      420
cgcacatcat ctctgctacc caagatgtcc cggctgtggn aagatgccan aaaangaacc      480
aangaactgc catactgncc actctgggtc tctgccaccc                            520
```

<210> 99

<211> 470
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(470)
<223> n = A,T,C or G

<400> 99
```
tttttttcaa agcaattgtg acacctacct gtggaccaag gaaaaaacca aatcatccnc    60
aatctttcag ttccacaaca acaccattat aaaaacgagt tgatctgaag tggttccaaa   120
tctttcttat cttcaagatc tatgattagt aattctgact cgttgcaaag caacatattt   180
tttgagatac tgttgtgagc cccgggagaa tgtcagagtc ctgtccactt ggacatgggt   240
ggaggggagg ttgggcaggg ctgataaaag actagtacgc ctcgtgtctc ttcagtctta   300
ttttgcaaac atcatctata aaggtttttg ctactccaag ttttggtagc ccaagctcat   360
caaagcatgt gtctattatg tgnctancat agtaaaaatg gctctaaatt gcatataaat   420
gcccagattt taataatcta ttgnttcana agaaaacaac agnanngngt              470
```

<210> 100
<211> 570
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(570)
<223> n = A,T,C or G

<400> 100
```
agtttttatt tttttctgta acgcccatcc tgcctgcagt agaccctttg ggcttaagan    60
gtcctacaga ttctaatagt ttcctctttg tagatgtgtt aacatattta aaatacttag   120
gacatggcct ggtacgtgac agatggtgtt taacatcatt gtcatatgaa gaacactctc   180
tgtgggccaa tggaggtgtg ccttaaaatt cttcatcttg cctgattttg tttgcataac   240
ttctggagag tctgtgtcct cctcatctag gccacctttc cattttttgt gaaagatgac   300
cttgtccctg cagtctccgc caggagtcac acagcttttc acagcagctg ccatctttaa   360
ttctttttcc tgagattcca gcaaggttgt gacattgtca ctttttttgtt ctagactctt   420
ttaaattttc tgcatttgcc tgaaaagcac ccctgtaaga atagatttct catggctcta   480
aaaattattc ccaagaatac cttacttggt tcaaaagcag actgtttctc ttcatttcat   540
ctcaaatcan acttctgggc aagatgttct                                    570
```

<210> 101
<211> 365
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(365)
<223> n = A,T,C or G

<400> 101
```
atcaataaac caaggtaatg cctnagggtt ccctcccagt cctcncatca gctctggcct    60
catcaccaag gtcacanagn acacagggga gggggaanac ccacncacac tccttggaat   120
gggtcctgtt atttatgctt ggngcncagn catattanaa gaaaaaaaaa agctttgtat   180
```

tntttttccn catatnatgg ntgctgttta cacaccctgc caatgcntta gcnntggaga 240
gctttttgca atatgnnggg gaaaggggag ggagggaatg aaagtgccaa agaaaacatg 300
tttttaanaa ctngggtttt atacaataga atgttttcta gcagaaaaaa aaaaaaaaaa 360
aaaaa 365

<210> 102
<211> 546
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(546)
<223> n = A,T,C or G

<400> 102
antgacacac tagccccctg ccgcctcggc atgaacagca tgggcaccat ggatggcacg 60
ctgatggtgg taggaagcan aagggctcct gggcagaaag ggcttggtnt gaggtgaaac 120
cccnccttca agcctgggat ggcctgaggc ctgggggccg ggctgccagc tntgggtagt 180
ntgtgggtgg agtganaatt tatggtgctt tttccgggcc tgctcatgga ccaatcagca 240
tgcacttcct ccccttntgag cccataaaaa ccctggattc agccagactt ggacanatgt 300
caatactacc aactgtggga agaagctacc cacttcagga ctccttgact cctcaggatg 360
acctgcctac anaaagaagc tacccactat gggtnttntn tntgctgana gctggatact 420
ngtcaggatg acctgcctgc anaaanagct acccnccatg ggtntcctnt gaactgttct 480
gttgcccaat aaagctcctn tcctttttgc tcnccctcca aaaaaaaaaa aaaaaaaaaa 540
aaaaaa 546

<210> 103
<211> 376
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(376)
<223> n = A,T,C or G

<400> 103
ctcaactccc ncttcacctt atgtaatgtc tgggcctgag atttctctct ggctttantt 60
tcttgatgtt cctgtgatat ggcttctgcc agcaatgaaa acaaggtcct tatagtagtn 120
ctccatggag tccagttcat cctggtggcg tctcctttgt tcatctcgct tttctttggc 180
atagtttctt aggtctcgta atctttgctt ttgaatgttt aaaccttctt caaacagttt 240
cttaaatatc atttcttccc gggtcctcat tctcatcatt tttgcacaca actgaactct 300
ataatcatca taatattttc gagcacgaac gatttgctgn cgatnttctt ttatctttga 360
ttgggncaac ctttgc 376

<210> 104
<211> 700
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(700)
<223> n = A,T,C or G

<400> 104
tttgaaaata cagattttta ccaactttgg attctttttt agttatatgt ttgtctttcc 60
tttttaaatt gttcaaaact atttttttaat ggtcaagtta ctaacacttg aaaatcagat 120
actgcaccaa atacagtgtt tttccgtagt gtttttaatg agtgcaccta ttactactgt 180
gcgagaattc atgttttacc agtcattgtt atattacaaa cagacttgca tgattaacca 240
gttgttacac ttactttttc aagttggagt atatatgact cagtgcagac tggtctctct 300
tatgtgaatg cacacatgca gaaatgcaga gtcaatttta catgcccata aagacatttg 360
taaagaattc agctcttatg gtctgttgta taaatgtgta tctaggcact ttggaatttg 420
acctcacaga tgttacaact tgatcagtcg tttgacctaa tttgtggnag ctatctgtat 480
gttttgcaat cttaatacag acatgctttc caaaaagatt aatacagaac catcctgccg 540
ttttggataa gtctatccag ctgtggaaag ggcaacctgt ggtttctctg tactggtgtt 600
taatgggga agaatatgaa cagctttaaa gagctgtgta ttgggggtac tactattaaa 660
aaataagatc tgcacgagtc tgactggcct ttgggtggcc 700

<210> 105
<211> 729
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(729)
<223> n = A,T,C or G

<400> 105
taaacccctc acactgagaa ctgcttgtgt gggagagagc tggttgggtt gatcttttcc 60
gagtgtgact tacctccttc aaggggatgt ttaagcttct cgggcagaag tggtgtgtct 120
attcctgaca ccaaacaccg tggtatatgt ggttgtcaca ctcagctagt gatgataaag 180
gtgttcttaa atatgttagc tttcagtttt cctgaggaag caattttatg gatacttccc 240
cctccttctc aagtgaggaa tagcagagca aattttattt ggaacttaaa ccaatagtta 300
taaccaatag tttcaacctc ctgcctcacc actgcttcct tcctgagctc tttccccaca 360
cctcaaaaag agtacaaagt gattccatct gcagaggtaa attctttgtt taaaaaagta 420
ctgtttttct tatcttttct ggttctccta ggtatcagaa caaggtttaa tagaaatcct 480
taaaaaagta agccaacaaa cagaaaagac aacaacagtg aaagtaagtg tccccanatg 540
cttgtggcaa atgaaaagat ggatacttta aagattaatg ttgagtatac atctaccaca 600
catatttttc agcccanaga cattttcctt ttgtcaaaca cgtgaaagtt tggggagaaa 660
ngctgaatct gttgggggag ggttctaatt tttataggct cttgactcca ttcccccctt 720
ttaattcac 729

<210> 106
<211> 481
<212> DNA
<213> Homo sapien

<400> 106
gaaaagcaga gtcagctact gagagctctg gaaagaagga tgtcaagaag gtgaaatcct 60
aaagcctaga aataaagttt taaatgggaa actgctattt tcttgttccc atcttcaaat 120
gctaattgcc agttccagtg tattcatggt actctaagaa aaatctcttt ggttttgatt 180
tcttgcatat tttatatatt ttacaatgct ttctacctga aatgtgtagc tttatatttt 240
atggcattct agtatttttg tgtactgtat tttgtgcatt tcatgtcttc atcaaaatcc 300
tctcagtcct tgttcttttg aagcttgtgc tgaggtttta gcttttctat gttttatatg 360
ccgctgcttt gaaagagaac ctagattcta tagttgtatt attgttgttt catactttaa 420
atttatatgg ctgtggaaaa acgaattaaa atgttttgag gagaaaaaaa aaaaaaaaaa 480
a 481

<210> 107
<211> 519
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(519)
<223> n = A,T,C or G

<400> 107
cagctcctct cctgccagag ctaggcaggc gccgaagtag ccgcatggcc ccgtcagant 60
accccaggga ctggagagcc aacctcaaag gcaccatccg tgagacaggc ctggagacca 120
gctccggtgg gaagctggct ggccatcaga agaccgtccc cacggctcac ctgacttttg 180
ttattgactg cacccacggg aagcagctct ccctggcagc aaccgcatca ccaccccaag 240
cccccagtcc caatcgaggg cttgtcaccc caccaatgaa gacctacatc gtgttctgtg 300
gggaaaactg gccccatctg actcgggtga cccccatggg tgggggatgc cttgcccagg 360
ccagggccac cctgccgctc tgcagagggt ctgtggcctc agcttccttc ccagtcagcc 420
cgctctgccc ccaggaggtt cccgaggcta aggggaaacc cgtgaaggct gcgcctgtga 480
ggtcttcaac ttggggaaca gtcaaggact cactgaaag 519

<210> 108
<211> 669
<212> DNA
<213> Homo sapien

<400> 108
ggacaatgaa gactgaacta tcgcacatta cctaagaaag atgggaattg acatgcacat 60
cacaattgta tacacaacag aaattattga atcatgagat atacattccg gtgtgtgaca 120
gattggcaca tgacataatc tgggttcttt atagactcag ttgttttggg gcgatctaga 180
ttatcaagag aagagccttc atgcttagct ttatcttgta ccaaccacca gggcccttgc 240
ttctgagcag gaagcagctg gggaataggc tctttctctt aatgacttcc aacatagttc 300
tctcaaacct tactcctcca gaaggccacc ctcacctggc tatggctact tcagaaaaaa 360
cttggcctct ggtataatag agcagaatca tcacctcaca ttctatttca agccaaagtc 420
aatatctcaa aggctggttc tgtgatttat ttggctcttg ggagctccta ctgaaagtgc 480
tgaaatgtcg tactgacact tcagacttat agctacctag actccaagta agatttatct 540
ctgactggag ggtttctcct attaaaaacc aaagagtgta gggtgccttc acctgctagg 600
taatcttcta tgccctaatg ggaagaatgg gagcagcaga caagtaagtg caggaaggag 660
aaccaaagc 669

<210> 109
<211> 349
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(349)
<223> n = A,T,C or G

<400> 109
tttcaagccg gattttgggc ctgcttaaac cacttaaatg tanttaatga cagatggttt 60
gaggtttaaa agtcttctgg agaaagcccg ccagagaaca ttccctttga agccccatgt 120
aaaaatacgt gtgggagaga aagtgttttc tctgacttct gctgacagtg gctaaaactc 180

tgaactgtca ggagtattca aaataagact gccttgtagg taagcctgtg gtagcttttt 240
tgagcacagg ataaaatact tgagtctttg cttaaatgtt actttctcaa tgaggctttg 300
tatgactaaa taaaatctgt ataatcccca aaaaaaaaaa aaaaaaaaa 349

<210> 110
<211> 337
<212> DNA
<213> Homo sapien

<400> 110
ggcctttccc actggtccat ctggttttct ctccagggtc ttgcaaaatt cctgacgaga 60
taagcagtta tgtgacctca cgtgcaaagc caccaacagc cactcagaaa agacgcacca 120
gcccagaagt gcagaactgc agtcactgca cgttttcatc tctagggacc agaaccaaac 180
ccacccttc tacttccaag acttattttc acatgtgggg aggttaatct aggaatgact 240
cgtttaaggc ctattttcat gatttctttg tagcatttgg tgcttgacgt attattgtcc 300
tttgattcca aataatatgt ttccttccct caaaaaa 337

<210> 111
<211> 552
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(552)
<223> n = A,T,C or G

<400> 111
attttgtcaa gtttctttaa tggctgaaca gaaagaagct tcaagtaatg gagaaggcat 60
tgtctgagtg cagctgcttt cctggacgcc tgtgccgttc ctgtcttcca aatcctatgc 120
ttggaggccc ctggaggtac atttttgcca ggaaccaacc tgaccttaaa aagatgagtg 180
tgacacagcc ggctgggcag gaggatggag gtgccacagg acaccacctg cccacgccct 240
ggccagcctg gccatgctgc cgagtgcggg gaggccaccc cacccagagg gcacagggca 300
aaccctaagc acgggggtat tgcccttgaa gccccagggg atgccctgtg ccggatcctc 360
atgcctcatt gactagcctg cttgctgaag gagcccaggg gcctgagcct gcaacactgc 420
aaggggtgag aagggcatgc tgctgtgggc gccactggac tcaaacctca cattanaagc 480
tacaaagaac cccaaatgcg cttcaanagc cccaccaacc cccaagccan gctcatncct 540
gacacantga at 552

<210> 112
<211> 115
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(115)
<223> n = A,T,C or G

<400> 112
gtcctaggta canaaacttc ccaaacaana actatgcncg ancnctaccc cttcgagtgc 60
tctncnttct gctccggttc ccatggnttg cccccattta taaggtacgc tggga 115

<210> 113
<211> 649

<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(649)
<223> n = A,T,C or G

<400> 113
gcctgattaa aaactaagca gaagtagttt taacanaaat actcatgaaa atgtttngaa 60
actgaaattt aaacaactgt aatattaagg aaaccagaat caataaatca ctgtcttgcc 120
agcacagcta cagagtaaca tgattcaggg gaggaaagtt ccttagagtt acttttataa 180
ttcttttttt tttcctctta ggtttanaaa tcttacaaat ttaaacttta tccttttaaa 240
attatttgaa cataatttag atattgtaag cttaaaatac aaatgtttat agataacctc 300
tttaccataa actaatccct ggcaagccat ggctctcttt tttttttggt gtttaaagcc 360
tgtaaacagt ttttctgaat gatcatgaac ttttcttggg tttagcacta ggatttagct 420
atgaagagag ctcataggct ttcaggtgct aattgagatc tgccctgtta gagtcttggg 480
gtgctagatt ggtcacattg acaccagtgg cagggaaggc atctatgagt ttgatgcttt 540
ttatcacaca cttcagntgt ttagaaagtt attaccaata cttttaaaca acactccaag 600
aaaatttgct atatttcttt ctcatcacta cagagagagt agatttccc 649

<210> 114
<211> 650
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(650)
<223> n = A,T,C or G

<400> 114
tggcgattgg tgttggcggt ctggctcagc tgggcagggg gtaactttac tgatttgggg 60
gtggttttta gtttaatttt tcttttctag cttcccatcg acggtcagtg cgcacgttgt 120
aatcagctga ggccatgtca ggagacggag ccacggagca ggcagctgag tatgtcccag 180
agaaggtgaa gaaagcggaa aagaaattag aagagaatcc atatgacctt gatgcttgga 240
gcattctcat tcgagaggca caggtttagt gatataggat tacatttcct tctctatggg 300
tccaatcaca ctacttggtt ctgcagtgaa taatattttc ataatcctaa cattgtaaat 360
gctgtttatt ggttttcaat tttagaatca acctatagac aaagcacgga agacttatga 420
acgccttgtt gcccagttcc ccagttctgg cagattctgg aaactgtaca ttgaagcaga 480
ggttactatt ttattttatt ttttcttata tcagtatttg cagcattcac tgtagtgata 540
gaaaacaaag ttangaacat agccaattan gacaaggagg atttaaatgt gtcttacctt 600
tatttgtaa aataggtata aaggagtaat taaaatgaat ttttgaaatt 650

<210> 115
<211> 403
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(403)
<223> n = A,T,C or G

<400> 115

gtattatgac tttaaaaacc ccattattga aaagtacctg acaaggcagc tcacgaancc 60
caggcctgtg atcctggacc cggcggaccc tacnngaaac ttgggtggng gagacccaan 120
gggnnggagg canctggcac angaggctga ggcctgnctg aattacccat gctttaagaa 180
tngggatggg nccccantga gctcctgnat tctgctggtg anacctcctg cttcctccct 240
gccattcatc cctgcccctc tccatgaagc ttganacata tanctggaga ccattctttc 300
caaanaactt acctnttgcc aaaggccatt tatattcata tagtgacang ctgtgctcca 360
tattttacag tcattttggn cacaatcgag ggttnctgga att 403

<210> 116
<211> 397
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(397)
<223> n = A,T,C or G

<400> 116
cgaaagaaaa aaagtgatat tggaccctgg aaagattttg aaacttgagt ggtttgataa 60
cccttctatg tattgtaggg agaaaaaaaa aagtttattt tattccactg tcctccctta 120
aaagcatcat ttgancaata aatgaatatt gtctttaaac caagggttag ggaattttcc 180
tctctctctc tctctcctct ctctttctgt tcaaagaact tcaaacattt gggaccacct 240
ggtattctgt attttcactg gccatattgg aagcagttct agttgcattg tattgagttg 300
tgctggcagt agtttccatg cctgtcaatg tatcatagtc ctttgttgcc cagataaata 360
aatatttgat acgctttaaa aaaaaaaaaa aaaaaaa 397

<210> 117
<211> 59
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(59)
<223> n = A,T,C or G

<400> 117
cacttatggg gacaatggga agactcttct tttncactgg actgtacctg gacntnnaa 59

<210> 118
<211> 751
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(751)
<223> n = A,T,C or G

<400> 118
catcaacata cccgctttat tgctgactca tgacaactaa tgggaagaca tggctcagat 60
gtgcagccac agtgagcttc tgaacatttc ttctcagact aagctcttac acacagttgc 120
agttgaaaga aagaattgct tgacatggcc acaggagcag gcagcttcct gcagacatga 180
cagtcaacgc aaactcatgt cactgtgggc agacacatgt ttgcaaagag actcagagcc 240

aaacaagcac actcaatgtg ctttgcccaa atttacccat taggtaaatc ttccctcctc 300
ccaagaagaa agtggagaga gcatgagtcc tcacatggaa acttgaagtc agggaaatga 360
aggctcacca attatttgtg catgggttta agttttcctt gaaattaagt tcaggtttgt 420
ctttgtgtgt accaattaat gacaagaggt tagatagaag tatgctagat ggcaaagaga 480
aatatgtttt gtgtcttcaa ttttgctaaa aataacccag aacatggata attcatttat 540
taattgattt tggtaagcca agtcctattt ggagaaaatt aatagttttt ctaaaaaaga 600
atttctcaa tatcacctgg cttgataaca tttttctcct tcgagttcct ttttctggag 660
tttaacaaac ttgttcttta caaatagatt atattgacta cctctcactg atgntatgat 720
attaagttct attgcttact ttggatttct a 751

<210> 119
<211> 591
<212> DNA
<213> Homo sapien

<400> 119
aggcttcttg tcacactgaa cacatccagc cacaggcacc agctggttgg gaccagcagc 60
ccccagcatc ctcttgcact ggctggcaca aaaagaaacc tgctgtatac cccccaaagt 120
gtccctttcc caattacctc tggggtctct tgctgcttgc ctctgctgct ctggactggg 180
agagcttctg tcctgtgctg catgggtatt tagactgtgg gggagatgcc ccttcttata 240
gcactggagg aggaaaacaa attcttgtcc ccctcagaat gagagtggct ctttctgatt 300
tgcaagggca ctatggtcag ggcaaaggca tggcccaggt gtttaagtac agggtgacgt 360
gtgcctatgc aatggggtgg taaggcaggc acgaagagtc caaaaaatct aggtggcctc 420
tcagctctgc cacctctagc tgcatgacct tgggcaagct atgtaacccc aattgcctgc 480
tccattaaag actgtgaagg tagaatgttt gtaaagctct taacagtatg taagccttca 540
ataaatttca gttttcccct tgttttcttg atcaaaaaaa aaaaaaaaaa a 591

<210> 120
<211> 652
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(652)
<223> n = A,T,C or G

<400> 120
attttgcagt acattaaaac tgaggcccag agatgtgatt tgcttgaggc cacacagcta 60
gatttttggt ggaagtgggc cttgaacaca gtgtactttc tgcagtttct gactgtaaaa 120
cccagtgtct gctctctgag ttccatttcc aagcccccct ccatcttgga cctatgtggt 180
ctccaccata ttcacacacc accgccacca cttgccaatg cctctcttaa agcaatatac 240
ccattcgttc tcttattggg aactggatgg atgaagcccc aaattcagcc ccacccacag 300
agaagccttc ctacactcag cctctgtcca cccttggcaa atctttcaag ctctctcctc 360
caggaaagtg gggccccaac tcagtcactc cacccccttc caggtccctg aggctggttc 420
tactgtatcc ccatcacctc cacaactcca ctcacccctg acggctccat ccacctcacc 480
agttggaagg cttgtggttt cagagaggag caatgctggt cagcgctgcc cagactccag 540
tgtttacaga tcaccagcat ttacaaccaa tccaatggcc agaagcctcc tctaaccana 600
aggagttctg aaggggcaga tgggggtgtg agtagtcggg gagtcgggat tg 652

<210> 121
<211> 407
<212> DNA
<213> Homo sapien

<400> 121
ctctttagta tgacactggc aactgacggc actgcggata tgtttggaat aaggttcaaa 60
agaagaggct gttctaagaa agactaccga gtaatcaatc acctcctcag agaaagtctg 120
ctcaggaaac tctcctccca ccagcccctg cacttggttc cttgcgttgc ctctggctgg 180
aagctgttgt ccccaacttc agaaagtgtc tgcagatttc acaggctctc tcttgggtca 240
agggaccagc tctgtgaaca cggcaagtaa caacagagag aaaacgtgag gagaaaagag 300
agaccgggat tttgaaatcc tgtttcctga atgccatctc accaggcaca cgaaagaata 360
aaattaagga atcaagaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa 407

<210> 122
<211> 752
<212> DNA
<213> Homo sapien

<400> 122
caaatccctc ctatccaggc ttttctgtct ctaatacccc aagcgttacc cctgctcttc 60
cctcattccc ggggctgcag gcgccctcta cagtcgcagc tgtcacacca ctacctgtgg 120
ctgccacagc cccatcccca gctccagtcc tcccaggatt cgcctcagca ttcagttcca 180
atttcaactc cgctcttgtt gcacaagccg gtttatcatc tggacttcaa gctgcaggca 240
gttctgtttt tccaggcctt ttgtccctcc cgggtatccc tgggtttcct cagaatcctt 300
cacaatcatc cttgcaagaa ttacagcata atgcggctgc acagtcagca ttgttacagc 360
aggtccattc agcttcggct ctggaaagct atccagctca gcctgatggg tttcctagtt 420
atccttcagc gccaggaaca ccatttttctt tgcaaccaag cctgtcccag agtgggtggc 480
agtgaatact tttaactttt attctccttc agagcaacat cagaattgcc tgagaactgc 540
aatgaacaat ctgacaaatg tgaagctggc caaaagtcgg aaaatgagaa tgagggtaat 600
cctggagaaa ttgtgacaac aatttgaaaa ttgtggttgc attttaaagt gtgaacactc 660
ccctatgtaa atatgctgac aataaattgg atggagaatg gtatttaaaa agtgtttgga 720
gacttttcac ctgtcctata aaaatttgaa tt 752

<210> 123
<211> 401
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(401)
<223> n = A,T,C or G

<400> 123
atgaaccaaa tagaaataag catgcaacat gaacagctgg aagagagttt tcaggaacta 60
gtggaagatt accggcgtgt tattgaacga cttgctcaag agtaaagatt atactgctct 120
gtacaggaag cttgcaaatt ttctgtacaa tgtgctgtga aaaatctgat gactttaatt 180
ttaaaatctt gtgacatttt gcttatacta aaagttatct atctttagtt gaatattttc 240
ttttggagag attgtatatt ttaaaatact gtttagagtt tatgagcata tattgcattt 300
aaagaaagat aaagcttctg aaatactact gcaattgctt cccttcttaa acagtataat 360
aaatgcttag ttgtgatnaa aaaaaaaaaa aaaaaaaaaa a 401

<210> 124
<211> 103
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature

<222> (1)...(103)
<223> n = A,T,C or G

<400> 124
aagataactt gctgggacca catgcctgat tgccacagct gtcatcacgt tatttaatga 60
acctagtgct gaagacagtg aaaagggtcc attgacngtg gcn 103

<210> 125
<211> 1024
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(1024)
<223> n = A,T,C or G

<400> 125
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cggcgcagag aggaggggct tgcgccccgt agaaatgtca atcagagcct ggacccctgc 120
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<212> PRT
<213> Homo sapien

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Arg Gly Ser Ser Gly Gly Arg Glu Glu Gly Ala Arg Arg Arg Glu Glu
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Gly Leu Ala Pro Arg Arg Asn Val Asn Gln Ser Leu Asp Pro Cys Ala
35 40 45
Pro Ala Pro Ala Pro Ala Ser Ala Leu Gln Lys Pro Lys Ile Trp Ser
50 55 60
Leu Ala Glu Thr Ala Thr Ser Pro Asp Asn Pro Arg Arg Ser Pro Pro
65 70 75 80
Gly Ala Gly Gly Ser Pro Pro Gly Ala Ala Val Ala Pro Ser Ala Leu
85 90 95

Gln Leu Ser Pro Ala Ala Ala Ala Ala Ala Ala His Arg Leu Val Ser
100 105 110
Ala Pro Leu Gly Lys Phe Pro Ala Trp Thr Asn Arg Pro Phe Pro Gly
115 120 125
Pro Pro Pro Gly Pro Arg Pro His Pro Leu Ser Leu Leu Gly Ser Ala
130 135 140
Pro Pro His Leu Leu Gly Leu Pro Gly Ala Ala Gly His Pro Ala Ala
145 150 155 160
Ala Ala Ala Phe Ala Arg Pro Ala Glu Pro Glu Gly Gly Thr Asp Arg
165 170 175
Cys Ser Ala Leu Glu Val Glu Lys Lys Leu Leu Lys Thr Ala Phe Gln
180 185 190
Pro Val Pro Arg Arg Pro Gln Asn His Leu Asp Ala Ala Leu Val Leu
195 200 205
Ser Ala Leu Ser Ser Ser
210

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<211> 507
<212> PRT
<213> Homo sapien

<400> 127
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20 25 30
Gly Gly Arg Ser Gly Pro Gly Ala Gly Ala Ser Glu Leu Ala Ala Ser
35 40 45
Gly Ser Leu Ser Asn Val Leu Ser Ser Val Tyr Gly Ala Pro Tyr Ala
50 55 60
Ala Ala Ala Ala Ala Ala Ala Ala Ala Gln Gly Tyr Gly Ala Phe Leu
65 70 75 80
Pro Tyr Ala Thr Glu Leu Pro Ile Phe Pro Gln Leu Gly Ala Gln Tyr
85 90 95
Glu Leu Lys Asp Ser Pro Gly Val Gln His Pro Ala Thr Ala Ala Ala
100 105 110
Phe Pro His Pro His Pro Ala Phe Tyr Pro Tyr Gly Gln Tyr Gln Phe
115 120 125
Gly Asp Pro Ser Arg Pro Lys Asn Ala Thr Arg Glu Ser Thr Ser Thr
130 135 140
Leu Lys Ala Trp Leu Asn Glu His Arg Lys Asn Pro Tyr Pro Thr Lys
145 150 155 160
Gly Glu Lys Ile Met Leu Ala Ile Ile Thr Lys Met Thr Leu Thr Gln
165 170 175
Val Ser Thr Trp Phe Ala Asn Ala Arg Arg Arg Leu Lys Lys Glu Asn
180 185 190
Lys Met Thr Trp Ala Pro Arg Ser Arg Thr Asp Glu Glu Gly Asn Ala
195 200 205
Tyr Gly Ser Glu Arg Glu Glu Asp Glu Glu Glu Asp Glu Glu Glu
210 215 220
Ser Lys Arg Glu Leu Glu Met Glu Glu Glu Glu Leu Ala Gly Arg Gly
225 230 235 240
Gly Gly His Gly Gly Arg Gly Ala Gly Arg Arg Arg Arg Asp Glu Glu

245 250 255
Ile Asp Leu Glu Asn Leu Asp Ser Ala Ala Ala Gly Ser Glu Leu Thr
260 265 270
Leu Ala Gly Ala Ala His Arg Asn Gly Asp Phe Gly Leu Gly Pro Ile
275 280 285
Ser Asp Cys Lys Thr Ser Asp Ser Asp Asp Ser Ser Glu Gly Leu Glu
290 295 300
Asp Arg Pro Leu Ser Val Leu Ser Leu Ala Pro Pro Pro Pro Pro Val
305 310 315 320
Ala Arg Ala Pro Ala Ser Pro Pro Ser Pro Pro Ser Ser Leu Asp Pro
325 330 335
Cys Ala Pro Ala Pro Ala Pro Ser Ser Ala Leu Gln Lys Pro Lys Ile
340 345 350
Trp Ser Leu Ala Glu Thr Ala Thr Ser Pro Asp Asn Pro Arg Arg Ser
355 360 365
Pro Pro Gly Ala Gly Gly Ser Pro Pro Gly Ala Ala Val Ala Pro Pro
370 375 380
Thr Leu Gln Leu Ser Pro Ala Ala Ala Ala Ala Ala Ala Ala Ala His
385 390 395 400
Arg Leu Val Ser Ala Pro Leu Gly Lys Phe Pro Ala Trp Thr Asn Arg
405 410 415
Pro Phe Pro Gly Pro Pro Ala Gly Pro Arg Pro His Pro Leu Ser Met
420 425 430
Leu Gly Ser Ala Pro Gln His Leu Leu Gly Leu Pro Gly Ala Ala Gly
435 440 445
His Pro Ala Ala Ala Ala Ala Ala Tyr Ala Arg Pro Ala Glu Pro Glu
450 455 460
Ser Gly Thr Asp Arg Cys Ser Ala Leu Glu Val Glu Lys Lys Leu Leu
465 470 475 480
Lys Thr Ala Glu Gln Pro Val Pro Arg Arg Pro Gln Met Arg Leu Asp
485 490 495
Ala Ala Leu Val Leu Ser Ala Leu Ser Ser Ser
500 505